Abbreviated Title: Fully-Human Anti-BCMA-CAR T Cells

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Title: A Phase I Clinical Trial of T Cells Expressing a Novel Fully-Human Anti-BCMA CAR for Treating Multiple Myeloma

NCI Principal Investigator: James N. Kochenderfer, M.D.

Surgery Branch
Center for Cancer Research
National Cancer Institute
National Institutes of Health
10 Center Drive, Rm. 6B17, MSC 1203
Telephone: 240-760-6062
Email: kochendj@mail.nih.gov

Investigational Agents:

Drug Name:	Anti-BCMA-CAR-transduced autologous peripheral blood lymphocytes (PBL)	Cyclophosphamide	Fludarabine
IND Number:	18246		
Sponsor:	Center for Cancer Research (CCR), NCI, NIH		
Manufacturer:	Department of Transfusion Medicine (DTM)	Generic	Generic
Supplier:	Department of Transfusion Medicine (DTM)	CC Pharmacy	CC Pharmacy

PRÉCIS

Background:

- Multiple myeloma (MM) is a malignancy of plasma cells.
- MM is nearly always incurable.
- T cells can be genetically modified to express chimeric antigen receptors (CARs) that specifically target malignancy-associated antigens.
- Autologous T cells genetically modified to express CARs targeting the B-cell antigen CD19 have caused complete remissions in a small number of patients with leukemia or lymphoma. These results demonstrate that CAR-expressing T cells have anti-malignancy activity in humans.
- B-cell maturation antigen (BCMA) is a protein expressed by normal plasma cells and the malignant plasma cells of multiple myeloma.
- BCMA is not expressed by normal cells except for plasma cells and some mature B cells.
- We have constructed a novel anti-BCMA CAR that can specifically recognize BCMA-expressing target cells in vitro and eradicate BCMA-expressing tumors in mice.
- This CAR has an antigen-recognition domain made up of a single fully-human heavy chain variable region.
- We hypothesize that anti-BCMA-CAR-expressing T cells will specifically eliminate BCMA-expressing MM cells in patients
- Possible toxicities include cytokine-associated toxicities such as fever, hypotension, and neurological toxicities. Elimination of normal plasma cells and unknown toxicities are also possible.

Objectives:

Primary

- Determine the safety and feasibility of administering T cells expressing an anti-BCMA CAR to patients with MM.

Eligibility:

- Greater than or equal to 18 years of age and less than or equal to age 73.
- Patients must have measurable MM defined as a serum M-protein ≥1.0 g/dL or a urine M-protein ≥200 mg/24 hours or an involved serum free light chain (FLC) level ≥10 mg/dL (provided FLC ratio is abnormal) or a biopsy-proven plasmacytoma of 2.0 cm or more in largest dimension, or greater than or equal to 30% bone marrow plasma cells.
- Patients must have previously received at least 3 different treatment regimens for MM.
- Patients must have prior exposure to an IMiD such as lenalidomide, and a proteasome inhibitor
- Patients must have a creatinine level of ≤ 1.5 mg/dL

- Patients must have a cardiac ejection fraction ≥ 50%.
- An ECOG performance status of 0-2 is required.
- Patients on any anticoagulant medications except aspirin are not eligible.
- No active infections are allowed.
- Absolute neutrophil count ≥ 1000/μL, platelet count ≥ 55,000/μL, hemoglobin ≥ 8g/dL
- ALT and AST ≤ 2.5-fold higher than the upper limit of normal.
- At least 14 days must elapse between the time of any prior systemic treatment (including corticosteroids) and the required leukapheresis.
- At least 14 days must elapse between the time of any prior systemic treatment (including corticosteroids) and initiation of protocol treatment.
- The patient's MM will need to be assessed for BCMA expression by flow cytometry or immunohistochemistry performed at the NIH. The myeloma must express BCMA. If unstained, paraffin-embedded bone marrow or plasmacytoma sections are available from prior biopsies, these can be used to determine BCMA expression by immunohistochemistry; otherwise patients will need to come to the NIH for a bone marrow biopsy or other biopsy of a plasmacytoma to determine BCMA expression. The sample for BCMA expression can come from a biopsy obtained at any time before enrollment.

Design:

- This is a phase I dose-escalation trial
- Patients will undergo leukapheresis
- T-cells obtained by leukapheresis will be genetically modified to express an anti-BCMA CAR
- Patients will receive a lymphocyte-depleting chemotherapy conditioning regimen with the intent of enhancing the activity of the infused anti-BCMA-CAR-expressing T cells.
- The chemotherapy conditioning regimen is cyclophosphamide 300 mg/m^2 daily for 3 days and fludarabine 30 mg/m^2 daily for 3 days. Fludarabine will be given on the same days as the cyclophosphamide.
- After the chemotherapy ends, the patients will have two days with no treatments and then receive an infusion of anti-BCMA-CAR-expressing T cells.
- The initial dose level will be 0.75x10^6 CAR+ T cells/kg of recipient bodyweight.
- The cell dose administered will be escalated until a maximum tolerated dose is determined.
- Following the T-cell infusion, there will be a mandatory 9-day minimum inpatient hospitalization to monitor for toxicity.
- Outpatient follow-up is planned for 2 weeks, and 1, 2, 3, 4, 6, 9, and 12 months after the CAR T-cell infusion. Afterwards, follow-up will be every six months up to at least 5 years.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

Determine the safety and feasibility of administering T cells expressing a novel fully-human anti-B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) with a heavy-chain-only antigen-recognition domain to patients with multiple myeloma (MM) and to determine a recommended phase 2 dose of these anti-BCMA CAR T cells

1.1.2 Exploratory Objectives

- Evaluate the *in vivo* persistence and phenotype of T cells expressing the anti-BCMA CAR.
- Assess for associations between toxicity and immunologic parameters including blood CAR T-cell and cytokine levels.
- Assess gene expression of infused CAR T cells and associations of CAR T-cell gene expression with CAR T-cell *in vivo* persistence and anti-myeloma activity.
- Assess anti-BCMA-CAR-expressing T cells for anti-MM activity post infusion.

1.2 BACKGROUND AND RATIONALE

1.2.1 Introduction

Multiple myeloma (MM) is a malignancy of plasma cells that is almost always incurable. New therapies are needed for MM. Regressions of MM occurring in the setting of allogeneic stem cell transplantation (alloHSCT), and particularly after allogeneic donor lymphocyte infusions (DLIs), provide evidence that cellular immune responses can have a clinically significant anti-myeloma effect, but alloHSCT is associated with a significant transplant-related mortality and by chronic graft-versus-host disease.[1,2] Many patients have obtained lengthy complete remissions of

lymphoma or chronic lymphocytic leukemia after infusions of autologous T cells that were genetically modified to express chimeric antigen receptors (CARs) targeting the B-cell antigen CD19.[3,4] Anti-CD19 CARs are now FDA-approved treatments. The lasting complete remissions of MM that sometimes occur in the setting of alloHSCT and the encouraging results obtained treating patients with anti-CD19-CAR-expressing T cells are rationales for attempting to develop CAR-T-cell therapies for MM. B-cell maturation antigen (BCMA) is a protein that is expressed by MM cells.[5] BCMA is also expressed by normal plasma cells and by some normal B cells, but BCMA is not expressed by other normal cells.[5] The very limited expression of BCMA in normal tissues makes BCMA a very promising target for CAR-T-cell therapies. We previously developed the first anti-BCMA CAR, and we have demonstrated that T cells expressing this CAR have BCMA-specific activity *in vitro* and *in vivo*.[5] Anti-BCMA-CAR-expressing T cells can eradicate MM tumors in mice.[5] We then conducted a phase I clinical trial of T cells expressing this first anti-BCMA-CAR. This is a protocol to evaluate a new CAR that differs from our previously-tested anti-BCMA CAR by having an antigen-recognition domain made up only of a fully-human heavy-chain variable domain. The new CAR also differs from the old CAR by having a 4-1BB rather than a CD28 costimulatory domain. The new anti-BCMA CAR trial will enroll patients with advanced MM persisting or progressing despite at least 3 prior therapies. Patients enrolled on the trial will receive a single cycle of chemotherapy that is designed to decrease endogenous lymphocyte counts because extensive evidence exists that depleting endogenous lymphocytes, and possibly other cells, with chemotherapy or total body irradiation dramatically increased the anti-tumor activity of adoptively transferred T cells.[6-8] After the lymphocyte-depleting chemotherapy, patients will receive an infusion of autologous anti-BCMA-CAR T cells. The amount of T cellswill escalate with sequential dose levels until a maximum tolerated dose (MTD) is determined. If at least 3 patients are treated on each dose level without an MTD being established, up to 12 additional patients can be treated at the maximum feasible dose. The maximum feasible dose is defined as the dose of CAR T cells that can be feasibly produced.

1.2.2 Multiple myeloma epidemiology and standard treatment

MM is a neoplasm of monoclonal plasma cells.[9,10] For MM to be diagnosed, a patient must have a proliferation of monoclonal plasma cells plus other abnormalities such as anemia, renal insufficiency, hypercalcemia, and lytic bone lesions.[10,11] MM is the second most frequently occurring hematologic cancer in the United States (U.S.).[10] An estimated 22,350 new cases of MM were diagnosed in the U.S. in 2013, and an estimated 10,710 patients died from MM in the U.S. in 2013.[12] Recent improvements in the therapy of MM have occurred.[9,10,13] These improved treatments have increased the median survival of patients with newly-diagnosed MM from 3 years to slightly over 5 years, although the prognosis of newly-diagnosed MM patients varies widely.[10,13]

Current standard non-transplant therapies for MM include various combinations of dexamethasone, bortezomib and its analogs, lenalidomide and its analogs, prednisone, melphalan, and cyclophosphamide.[9,10,13,14] Myeloablative doses of chemotherapy followed by autologous stem cell transplantation is a standard therapy for MM patients with good performance status, and adequate bone marrow stem cells.[10,13] Compared to standard doses of chemotherapy for first treatment of MM, administration of myeloablative doses of chemotherapy followed by autologous stem cell transplantation improved progression-free survival in most trials and overall survival in some trials.[10,13] Myeloablative chemotherapy followed by autologous transplantation can also be used as a treatment for relapsed MM in some cases.[10,13,15] Use of the International Uniform

Response Criteria for Multiple Myeloma is the most common approach for assessing clinical outcomes in MM.[16] Despite the recent improvements in treatment, MM remains an almost always incurable disease.[13,17,18] Patients obtaining remissions of MM almost always relapse.[13] The median overall survival of patients with relapsed MM is 3 years or less.[17] Survival is shorter for patients treated with lenalidomide plus dexamethasone who have received at least 2 prior lines of therapy compared to patients who received only one prior line of therapy.[19] In patients with MM that was refractory to bortezomib, the median overall survival was only 9 months when the patient was also either ineligible for thalidomide or lenalidomide, or the patient had MM that was relapsed or refractory to thalidomide or lenalidomide.[18] Utilizing the immune system to treat MM is one possible way to improve therapy of MM. So far, the clearest evidence of activity of the immune system against MM comes from allogeneic transplantation studies.

1.2.3 Allogeneic transplantation for MM

Allogeneic stem cell transplantation (alloHSCT) can cure a fraction of patients with MM.[20,21] Myeloablative alloHSCT can induce long-term complete remissions of MM, but also has a transplant-related mortality rate (TRM) of 20% to 50%.[20,21] The high TRM of myeloablative alloHSCT led investigators to test nonmyeloablative alloHSCT for MM.[20-22] A commonly used transplantation strategy is to administer myeloablative chemotherapy plus an autologous stem cell transplant and then to conduct a nonmyeloablative alloHSCT a short time later.[1,20,21] In some studies, this strategy has been shown to yield higher rates of progression-free and overall survival when compared to the strategy of 2 sequential autologous stem cell transplants.[1,22] One recent trial showed an 8-year progression-free survival rate of 22% for patients receiving an autologous transplant followed by a nonablative alloHSCT compared to an 8-year progression-free survival of 12% for patients receiving sequential autologous transplants.[1] These results showed a statistically significant advantage for the autologous followed by alloHSCT strategy, but also point out that the vast majority of patients are not cured by either approach.

Because many nonmyeloablative transplant regimens that have been used to treat MM include very low doses of radiation or chemotherapy, nonmyeloablative alloHSCTs depend on an immunologic graft-versus-myeloma effect, and remissions of MM that occur after nonmyeloablative alloHSCT provide evidence that immune responses can be effective at eliminating MM.[1,20,21] Direct evidence that lymphocytes can eliminate MM comes from donor lymphocyte infusions (DLIs).[2,21] Twenty-two to twenty-eight percent of patients receiving DLIs to treat persisting MM after alloHSCT have achieved complete remissions (CRs).[2,20,21] Of note, some of these patients obtaining CRs after DLIs did not receive any other therapies around the time of their DLI.[2] These results demonstrate the ability of the immune system to eradicate MM. Unfortunately, both alloHSCT and DLIs utilize allogeneic lymphocytes; therefore, they are associated with the sometimes fatal complication of graft-versus-host disease[2,20,21]; in addition most patients receiving DLIs for MM do not obtain CRs, so developing of an effective autologous cellular immune therapy for MM would be a major advance.

1.2.4 T-cell gene therapy

T cells can be prepared for adoptive transfer by genetically modifying the T cells to express receptors that specifically recognize tumor-associated antigens.[23-30] Genetic modification of T cells is a quick and reliable process, and clinical trials of genetically modified T cells targeting a variety of malignancies have been carried out.[3,4,27,31,32] Genetically modified antigen-specific T

cells can be generated from peripheral blood mononuclear cells (PBMC) in sufficient numbers for clinical treatment within <10 days.[3] Genetically modifying T cells with gammaretroviruses consistently causes high and sustained levels of expression of introduced genes without in vitro selection[4,31,33,34], and genetic modification of mature T cells with gammaretroviruses has a long history of safety in humans.[35-37] There are two general approaches for generating antigen-specific T cells by genetic modification: introducing genes encoding natural αβ T cell receptors (TCRs) or introducing genes encoding CARs.[24,26,27,29] CARs are fusion proteins incorporating antigen recognition moieties and T cell activation domains.[28,38-40] The antigen-binding domains of most CARs currently undergoing clinical and preclinical development are antibody variable regions.[24,28,38,40] TCRs recognize peptides presented by human leukocyte antigen (HLA) molecules; therefore, TCRs are HLA-restricted, and a particular TCR will only be useful in patients expressing certain HLA molecules[24,26,27,38], which limits the number of patients who could be treated with T cells genetically modified to express a TCR. In contrast, CARs recognize intact cell-surface proteins and glycolipids, so CARs are not HLA-restricted, and CARs can be used to treat patients regardless of their HLA types.[24,27,41-43]

1.2.5 Chimeric antigen receptors

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.[44,45] These experiments led to a clinical trial of CAR-transduced T cells targeting the α-folate receptor on ovarian cancer cells; no tumor regressions were observed during this clinical trial.[46] CARs that were capable of recognizing a variety of tumor-associated antigens have been evaluated in many centers.[24,38] Preclinical studies have assessed a wide variety of factors that could affect in vivo function of CAR-expressing T cells. Multiple approaches for inserting CAR genes into T cells by using gammaretroviruses[4,31,33-35,47-49], lentiviruses[3,50-53], or transposon systems[54,55] have been assessed. Because all methods of T-cell genetic modification require a period of in vitro culture, various T-cell culture techniques have been evaluated.[3,47,56] Different portions of CARs including antigen-recognition moieties, extracellular structural components, costimulatory domains such as the cytoplasmic portion of the CD28 protein, and T-cell-activation moieties such as the signaling domains of the CD3ζ protein can all be important to the in vivo function of CAR-expressing T cells, and all of these portions of CARs remain the subject of intensive investigation.[38,47,51,57-59] Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.[7,47-49,54,60,61] Data suggesting that T-cell costimulation played an important role in the activity of CAR-expressing T cells in vivo led investigators to add signaling moieties from the costimulatory molecule CD28 to CARs.[49,58] These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.[58,62,63] T cells expressing CARs with CD28 signaling moieties and CD3ζ signaling domains were more effective than T cells expressing CARs without CD28 moieties at eradicating human leukemia cells from mice.[62,63] Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.[50] Anti-CD19 CARs containing the signaling domains of both 4-1BB and CD3ζ were superior to CARs containing the signaling domains of CD3ζ without any costimulatory domains at eradicating human malignant cells from mice.[51,57] Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced *in vivo* persistence.[51] In contrast to T cells expressing a CAR with a CD28 moiety, the increased in vitro proliferation and prolonged in vivo persistence of T cells expressing a 4-1BB-

containing CAR occurred whether or not the T cells were exposed to the antigen that the CAR recognized.[51,57]

Results from several clinical trials of anti-CD19 CAR T cells have been reported to date in peer-reviewed papers.[3,4,31,33,34,64-67] The first evidence of antigen-specific activity of anti-CD19 CAR T cells in humans was generated during a clinical trial at the National Cancer Institute (NCI) in a patient who experienced a dramatic regression of advanced follicular lymphoma.[33] This clinical trial utilized a gammaretroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3ζ molecules.[33] The first patient treated on this protocol had a large disease burden of follicular lymphoma. This first patient experienced no acute toxicities except for a low grade fever that lasted for 2 days, and he obtained a partial remission (PR) that lasted for 32 weeks.[33] Bone marrow biopsies revealed a complete elimination of extensive bone marrow lymphoma that was present before treatment; in addition, normal B-lineage cells were completely eradicated from the bone marrow.[33] The bone marrow B-cell eradication was confirmed by flow cytometry, and it persisted for over 36 weeks.[33] B cells were also completely absent from the blood during this time, while T cells and other blood cells recovered rapidly.[33] Seven months after the anti-CD19 CAR T cell infusion, progressive lymphoma was detected in the patient's cervical lymph nodes. The lymphoma remained $CD19^+$, so the patient was treated a second time with anti-CD19 CAR T cells. The first and second treatment regimens were the same except the patient received a higher dose of cells with the second treatment. After the second treatment, the patient obtained a second ongoing partial remission.[4]

Seven more patients were subsequently treated with the same regimen of chemotherapy, anti-CD19 CAR T cells, and high-dose IL-2.[4] In 4 of 7 evaluable patients on the trial, administration of anti-CD19 CAR T cells was associated with a profound and prolonged B-cell depletion.[4,33] In all 4 patients with B-cell depletion, the depletion lasted for over 36 weeks. The B-cell depletion could not be attributed to the chemotherapy that was administered because blood B-cells recovered to normal levels in 8 to 19 weeks in patients receiving the same chemotherapy plus infusions of T cells targeting NY-ESO or gp100, which are antigens that are not expressed by B cells.[33] Because normal B cells express CD19, prolonged normal B-cell depletion after anti-CD19 CAR T-cell infusions demonstrated that CAR-expressing T cells had a powerful ability to eradicate $CD19^+$ cells in humans. All of the patients with long-term B-cell depletion obtained either complete or partial remissions of their malignancies, and the 4 patients with long-term B cell depletion also developed hypogammaglobulinemia. Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins. Of the eight patients treated, seven patients were evaluable for malignancy response; the one patient who was not evaluable died with pneumonia caused by influenza A.[4] Six of the seven evaluable patients had remissions of their malignancies. Two of the remissions were complete remissions (CRs) of CLL.[4] Both of these CRs were confirmed by multicolor flow cytometry of bone marrow cells.[4] One of these CRs lasted 24 months, and the other is ongoing at 30 months.[4] Most patients treated with this regimen of chemotherapy, anti-CD19 CAR T cells, and IL-2 experienced significant acute toxicities including fever, hypotension, and neurological toxicities such as delirium and obtundation.[4] All of these toxicities peaked within 10 days after the cell infusion and resolved less than 3 weeks after the cell infusion.[4] These acute toxicities correlated with serum levels of the inflammatory cytokines tumor necrosis factor and interferon-γ, and T cells producing these inflammatory cytokines in a CD19-specific manner were detected in the blood of patients after the anti-CD19 CAR T cell infusions.[4] Subsequent studies with this same CAR demonstrated the effectiveness of anti-CD19 CAR T cells

against diffuse large B-cell lymphoma and led to multicenter phase II trials and an application for FDA approval.[66]

1.2.6 BCMA

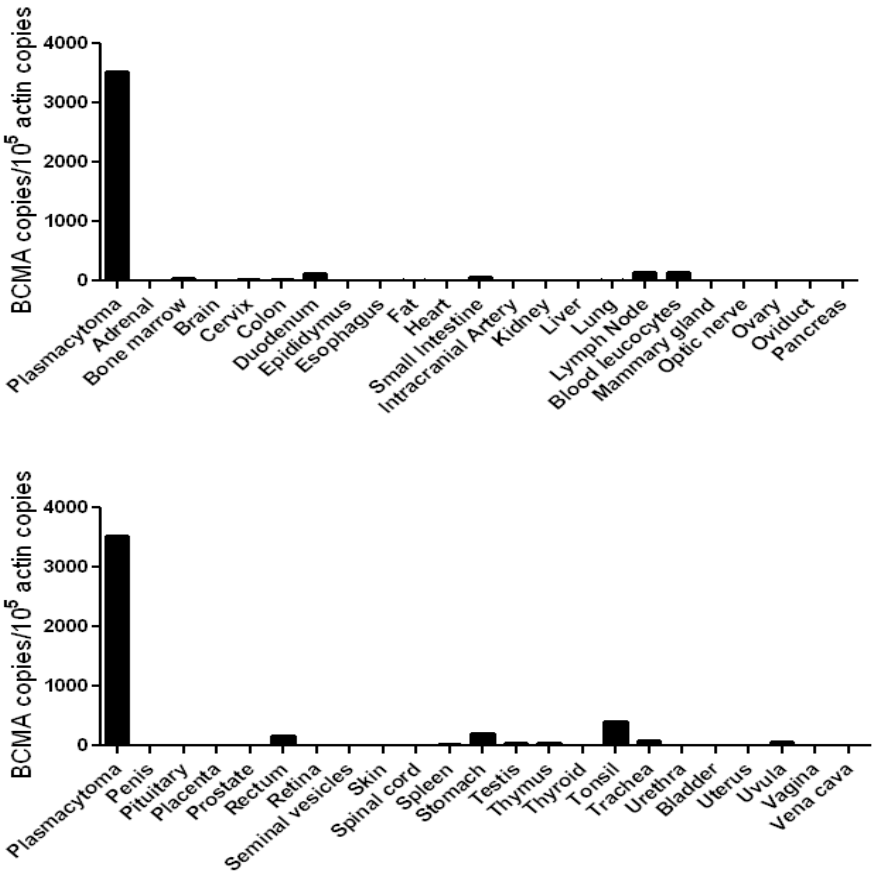
One candidate antigen for immunotherapies for MM is BCMA (CD269) [68,69]. BCMA RNA was detected universally in MM cells, and BCMA protein was detected on the surface of plasma cells from MM patients by several investigators [70-73]. BCMA is a member of the tumor necrosis factor receptor (TNF) superfamily [74,75]. BCMA binds B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) [75-77]. Among nonmalignant cells, BCMA has been reported to be expressed mostly by plasma cells and a small subset of mature B cells [68,69,76,78,79]. Mice deficient in BCMA were healthy and had a normal physical appearance [80,81]. BCMA-deficient mice had normal numbers of B cells, but survival of long-lived plasma cells was impaired [78,80].

A critical factor for any antigen being considered as a target for immunotherapies is the antigen's expression pattern in normal tissues. BCMA has been reported to be expressed in plasma cells and in some B cells but to otherwise have limited expression [68-70,79]. To more completely assess the expression pattern of BCMA, we performed quantitative polymerase chain reaction (qPCR) on a panel of cDNA samples from a wide range of normal tissues (**Figure 1**).[5] As a positive control, we performed qPCR on cDNA from cells of a plasmacytoma that was resected from a patient with advanced MM.[5] Ninety-three percent of the cells from the plasmacytoma sample were plasma cells as determined by flow cytometry. The BCMA expression of the plasmacytoma sample was dramatically higher than the BCMA expression of any other tissue (**Figure 1**). Not surprisingly, BCMA cDNA copies were detected in several hematologic tissues such as blood leukocytes, bone marrow, spleen, lymph node, and tonsil. Low levels of BCMA cDNA copies were detected in the samples of testis and trachea. In addition, low levels of BCMA cDNA copies were detected in most gastrointestinal organs such as duodenum, rectum, and stomach (**Figure 1**). One possible explanation for BCMA expression in gastrointestinal organs and the trachea was the known presence of plasma cells and B cells in tissues such as lamina propria and Peyer's Patches [82,83].

In **Figure 1**, actin cDNA copies and BCMA cDNA copies were measured by qPCR in all of the samples, and the results were expressed as the number of BCMA cDNA copies per 10^5 actin cDNA copies.[5]

Figure 1

Figure 1



After demonstrating a very restricted expression pattern of BCMA RNA by qPCR, we carried out an assessment of BCMA protein expression by immunohistochemistry (IHC). As expected, our anti-BCMA IHC staining procedure yielded strong staining of BCMA-K562 cells, which expressed high levels of BCMA after being transduced with the gene encoding BCMA. We found a lack of BCMA staining with NGFR-K562 negative control cells.[5] We went on to evaluate BCMA protein expression in normal human organs. Except for plasma cells, we did not detect BCMA protein expression by the cells of any of the organs that we stained. We detected plasma cells expressing cell-surface BCMA in gastrointestinal organs. BCMA expression by normal plasma cells probably accounts for the low levels of BCMA RNA detected in these organs because we did not detect BCMA expression by any of the other cells in these organs. We detected BCMA-expressing plasma cells in the tonsil. The organs assessed by IHC and found to lack BCMA expression except for plasma cells are shown in **Table 1**[5] We concluded that BCMA expression detected at low levels in some organs by qPCR (**Figure 1**) was due to plasma cells in these organs.

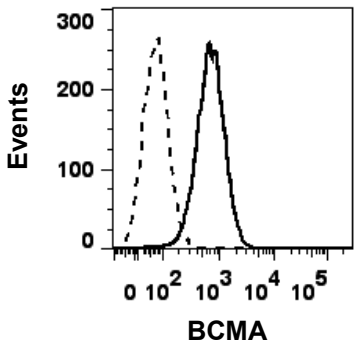
Table 1

Organs stained for BCMA by immunohistochemistry and found to lack of BCMA expression except on plasma cells

adrenal	lung
bladder	ovary
bone	pancreas
breast	parathyroid
cerebellum	pituitary
cerebral cortex	placenta
duodenum	prostate
eye	skin
fallopian tube	spinal cord
esophagus	spleen
stomach	skeletal muscle
small intestine	testis
colon	thymus
rectum	thyroid
heart	trachea
kidney	cervix
liver	uterine endometrium

For a protein to be an appropriate target for CAR-expressing T cells aimed at MM, the protein must be expressed on the surface of MM cells. By flow cytometry of bone marrow samples from patients with MM, we found that 6/10 tested samples uniformly expressed high levels of BCMA by flow cytometry. An example of this flow cytometry staining is shown in **Figure 2**.

Figure 2



As shown in **Figure 2** flow cytometry for BCMA (solid line) and isotype-matched control staining (dashed line) revealed BCMA expression on the surface of MM cells from a plasmacytoma of a patient with MM.[5] The plot is gated on plasma cells, which made up 93% of the total plasmacytoma cells. In addition to the flow cytometry studies, we stained tissue sections from plasmacytomas of 3 patients with MM. In all 3 of the samples, the neoplastic plasma cells expressed cell-surface BCMA.[5]

1.2.7 Previous clinical experience with anti-BCMA CAR T cells

This pre-clinical work led to the first in-humans clinical trial of CAR-Ts targeting BCMA at the NCI.[84] An anti-BCMA CAR containing a murine scFv, a CD8 hinge and transmembrane region, a CD28 costimulatory domain, and a CD3 T-cell activation domain was created by synthesizing the appropriate DNA and ligating it into a gamma-retroviral backbone.[84] Patients who had MM with uniform BCMA expression by either IHC or flow cytometry were enrolled.[84] All participants received conditioning chemotherapy prior to infusion of CAR T cells with goals of depleting endogenous leukocytes, including depletion of regulatory T cells, and increasing serum cytokine levels of IL-15 and IL-7 to increase CAR-T activity.[84-87] The chemotherapy regimen used was cyclophosphamide 300 mg/m^2 and fludarabine 30 mg/m^2 given daily on days -5 to -3 followed by an infusion of CAR-BCMA T cells on day 0.[84] Twenty-six patients have received CAR-BCMA T cells treated with either 0.3x10^6, 1x10^6, 3x10^6, or 9x10^6 CAR+ T cells/kg of bodyweight; 16 patients were treated on the highest dose level. Five patients had a history of poor-prognosis chromosome 17p-deletion prior to protocol enrollment; results of 10 of these patients have been published.[84] Toxicities were mild in patients receiving lower doses of CAR-Ts (0.3-3.0x10^6 CAR-T/kg).[84] As the dose of CAR-T escalated, patients began to have more symptoms of cytokine release syndrome.[84] Patients treated at the highest dose level (9x10^6 CAR$^+$T-cells/kg) experienced cytokine-release syndrome and a variety of grade 3 and 4 toxicities including neutropenia, thrombocytopenia, hypotension, and acute kidney injury that were managed without long-lasting complications.[84] While CAR-BCMA T-cell toxicity was severe in some patients treated at the highest dose level, toxicities were mainly limited to the first two weeks after CAR-BCMA T-cell infusion. Because of Grade 3 and 4 CRS experienced by some patients with high bone marrow myeloma burdens, the clinical protocol was modified to only allow enrollment of patients with lower myeloma burdens defined as MM making up 30% or less of bone marrow cells. Two patients experienced delayed neutropenia and thrombocytopenia that started approximately one month after CAR T-cell infusion when blood counts had recovered from the chemotherapy administered before CAR-BCMA T-cell infusions. These patients were treated with filgrastim, eltrombopag, and prednisone based on the hypothesis that the cytopenias were caused by CAR T cells in the patient's bone marrow. At the time of delayed cytopenias, 15 percent of the first patient's bone marrow T cells were CAR+, and 16.5 percent of the second patient's bone marrow T cells were CAR+. In both cases, cytopenias resolved after approximately 1 month. A third patient had milder delayed thrombocytopenia and neutropenia that were treated with eltrombopag and G-CSF without corticosteroids.

Of the 10 patients treated with lower doses of anti-BCMA CAR-Ts, one patient experienced a VGPR that lasted eight weeks, eight patients had SD that lasted from two to 12 weeks and one patient had a transient PR.[84] Partial loss of BCMA expression on MM cells was demonstrated in one patient.[84] 13 of 16 evaluable patients treated at the highest dose level obtained objective anti-myeloma responses with 2 stringent complete responses, 8 very good partial responses, and 3 partial responses; the duration of responses varied. The longest response to date is 75 weeks. Eight of 10 evaluable patients treated with the highest dose of CAR-BCMA T cells obtained minimal residual disease negative status by bone marrow flow cytometry. Consistent with BCMA-specific T-cell activity, plasma cells were reduced on bone marrow core biopsies in all 8 evaluated patients and absent in 4 of these patients 2-3 months after CAR-BCMA T-cell infusion. Of the five patients who had a history of the poor-prognosis chromosome 17p-deletion prior to protocol enrollment,

four obtained a PR or better. These results demonstrate that CAR-BCMA T cells can induce responses in poor-prognosis MM.

A common concern raised with CAR-T therapy is whether soluble antigen may hinder efficacy. In the NCI trial, serum BCMA protein did not eliminate CAR-T cell activity against MM; moreover, serum BCMA levels were observed to drop significantly in patients who obtained responses to anti-BCMA CAR T cells.[84] In agreement with pre-clinical work, the phase I clinical trial with anti-BCMA CART cells did not demonstrate undue damage to nonhematopoietic organs after infusions of T-cells.[84,88]

Cohen *et. al* at the University of Pennsylvania have published their initial findings from a phase I trial of anti-BCMA CAR-T without conditioning chemotherapy in patients with relapsed or refractory MM.[89] One patient was treated with $1.8x10^8$ CAR T cells, two with $2x10^8$ CAR T cells and three patients at the highest dose level of $5x10^8$ CAR T cells.[89] Toxicities were similar to those published by Ali *et. al* from the NCI experience, except for an episode of grade 4 posterior reversible encephalopathy syndrome (PRES) that manifested as severe delirium, seizures, obtundation and cerebral edema.[89] This was treated with steroids, anti-epileptics, and cyclophosphamide; there was no long-term neurological dysfunction.[89] Reported results show one patient has an ongoing stringent CR at seven months with an MRD-negative bone marrow by flow cytometry.[89] One patient who had pleural and dural MM involvement was found to have CAR-Ts cells in pleural fluid and CSF and achieved VGPR with resolution of extramedullary disease; this patient's VGPR lasted 5 months, and progression was associated with loss of BCMA expression on MM cells.[89] Four patients had modest to minimal in vivo CAR T-cell proliferation and minimal to no anti-myeloma response.[89] Of note, the trial reported by Cohen *et al.* utilized an anti-BCMA CAR with fully-human antibody variable regions. Full trial methods and results have yet to be published.

A third anti-BCMA CAR-T clinical trial is a multicenter trial being conducted by bluebird bio Inc.[90] Twenty-one patients with relapsed/refractory MM have been infused with bb2121, an anti-BCMA CAR-T that expresses a CAR with the same scFv as the anti-BCMA CAR used at the NCI.[84] In contrast to the NCI anti-BCMA CAR, bb2121 has a 4-1BB costimulatory motif, and it is encoded by a lentivirus.[90] Bb2121 is administered after cyclophosphamide plus fludarabine lymphocyte-depleting chemotherapy.[90] To date, the authors report that the safety profile of bb2121 has been favorable through doses as high as $800x10^6$ CAR-Ts with no DLT observed.[90] Seventy-one percent of patients have had CRS that was generally mild.[90] Efficacy of bb2121 in this study has been promising. Of 3 patients receiving a CAR T-cell dose of $50x10^6$ CAR T cells, one patient obtained a PR, but no patients obtained responses of VGPR or CR.[90] Among 18 patients receiving CAR T-cell doses greater than 50 $x10^6$ CAR-Ts, 15 patients have reached at least 60 days of follow-up, and 11 of 15 patients with at least 60 days of follow-up have obtained best responses of VGPR or CR.[90] All patients with at least 60 days of follow-up receiving a cell dose of greater than 50 $x10^6$ CAR-Ts have obtained at least a PR.[90]

Fan *et al.* have reported early results from an on-going phase I anti-BCMA CAR trial that is enrolling patients with relapsed or refractory MM.[91] The CAR used in this study is called LCAR-B38M. Among 19 patients reported, there was a 100% overall response rate.[91] Eighteen patients obtained a stringent CR or a VGPR after receiving anti-BCMA CAR-T.[91] CRS occurred in 74% of patients but was noted to be mild in most patients.[91]

1.2.8 Anti-BCMA CAR development and preclinical testing

The anti-BCMA CAR to be assessed in this protocol contains an antigen recognition domain made up only of a single fully-human variable heavy chain (FHVH) domain rather than the traditional CAR antigen-recognition domain of a single-chain variable fragment (scFv). The FHVH33 heavy-chain variable region sequence was obtained as part of a research agreement with TeneoBio Inc. TeneoBio derived this heavy chain variable region by immunizing a proprietory rat that is transgenic for human heavy chain variable region domains. TeneoBio then selected heavy chain variable domains that bound with high affinity to human BCMA. Investigators in the laboratory of James Kochenderfer then generated 9 different CARs that each incorporated one of 4 different heavy-chain-only anti-BCMA CARs with different costimulatory domains. These CARs were assessed for specificity of antigen recognition and a wide variety of in vitro and in vivo functions. From these experiments the FHVH33 heavy-chain domain was found to be the one with the most sensitive and specific functional recognition of BCMA. After selecting FHVH33, the optimal overall CAR design was determined for evaluation in a clinical trial. The selected anti-BCMA CAR contains the hinge and transmembrane regions of the human CD8α molecule, the signaling moiety of the 4-1BB costimulatory molecule, and the signaling domains of the CD3ζ molecule. The CAR is designated FHVH33-CD8BBZ (**Figure 3**). Some of the experiments demonstrating the function of FHVH33-CD8BBZ are described in this section of this protocol. The clinical gene therapy vector encoding FHVH33-CD8BBZ is MSGV, which has been used in many prior clinical trials at the NCI.[66,92]

Figure 3

After transductions, we found high levels of cell surface expression of the FHVH33-CD8BBZ anti-BCMA CAR on the transduced T cells as shown in the representative example in **Figure 4**.

Figure 4

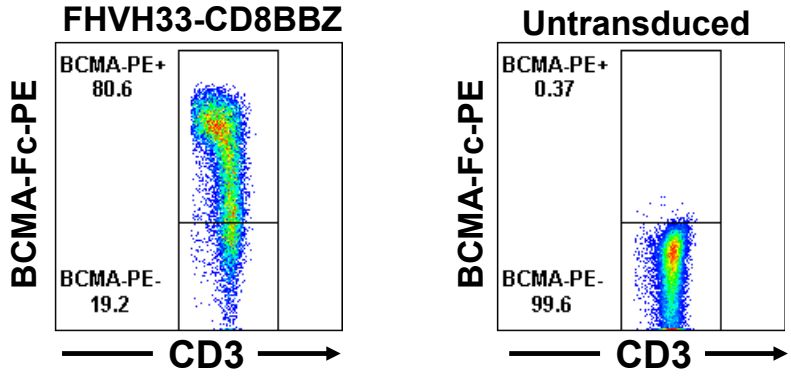


Figure 4 shows anti-BCMA CAR expression on T cells from a multiple myeloma patient 7 days after transduction with gammaretroviruses encoding the anti-BCMA CAR. Transductions were carried out 2 days after the cultures were started, so the T cells had been in culture for a total of 7 days at the time of this analysis. The plots are gated on live, $CD3^+$ lymphocytes.

We also performed a series of in vitro assays to assess the function of anti-BCMA-CAR-expressing T cells, and we found that FHVH33-CD8BBZ-CAR-expressing T cells exhibited BCMA-specific activities including CD107a upregulation and cytokine production in vitro. These experiments showed that anti-BCMA-CAR-expressing T cells are activated in a BCMA-specific manner.

Figure 5

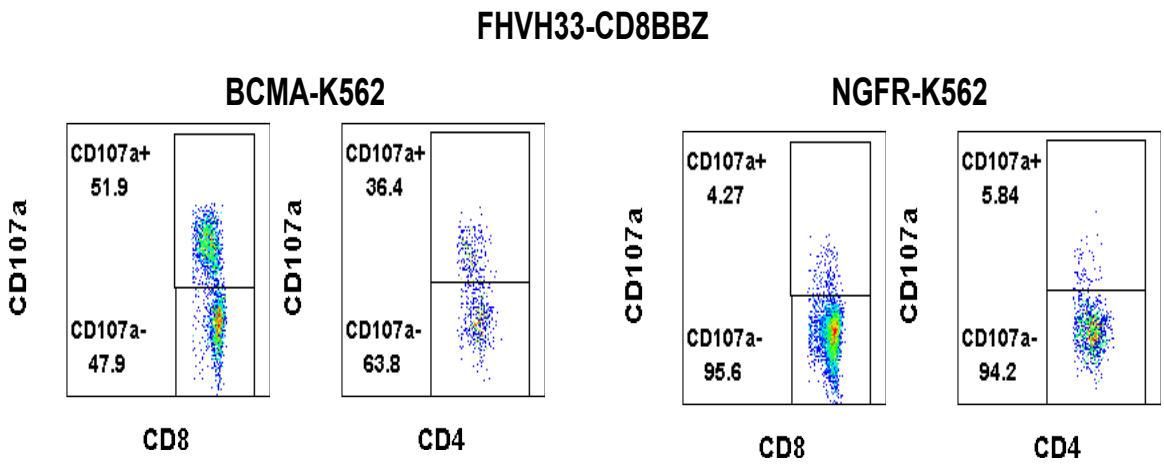
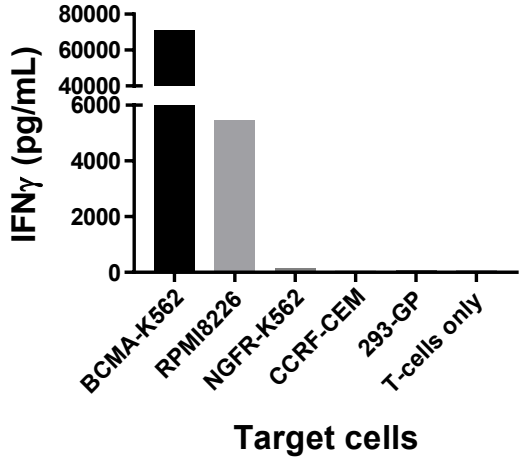


Figure 5 shows upregulation of CD107a, which indicates degranulation and correlates with cytotoxicity[93] when anti-BCMA CAR-expressing T cells were cultured with the BCMA-expressing cell line BCMA-K562. CD107a was not upregulated when anti-BCMA-CAR-expressing T cells were cultured for 4 hours with the BCMA-negative control cell line NGFR-K562. Untransduced T cells did not upregulate CD107a when cultured with either BCMA-K562 or NGFR-K562 (not shown). The plots are gated on live $CD3^+$ lymphocytes. T cells transduced with the FHVH33-CD828Z anti-BCMA CAR produced large amounts of IFNγ when they were cultured overnight with the BCMA-expressing cell lines BCMA-K562 and RPMI8226 (**Figure 6**). In contrast, the anti-BCMA CARs produced only background levels of IFNγ when they were cultured with the BCMA-negative target cell lines NGFR-K562, CCRF-CEM, and 293-GP. The anti-BCMA-CAR-transduced T cells also made minimal IFNγ when cultured without any target cell. The experiment depicted in **Figure 6** consisted of culturing the anti-BCMA-CAR-transduced T cells with the indicated target cell lines overnight and then performing a standard IFNγ enzyme-linked immunosorbant assay (ELISA) to detect IFNγ in the culture supernatant.

Figure 6



A second ELISA experiment performed in a similar manner utilizing FHVH33-CD8BBZ anti-BCMA CAR T cells from a different patient is shown in **Table 2**. In the experimental results shown in **Table 2**, three different types of effector T cells from the same multiple myeloma patient were evaluated, untransduced T cells, T cells expressing the FHVH33-CD828Z CAR, and T cells expressing the FHVH33-CD8BBZ CAR. FHVH33-CD828Z is identical to FHVH33-CD8BBZ except that FHVH33-CD828Z has a CD28 costimulatory domain while FHVH33-CD8BBZ has a 4-1BB costimulatory domain. The untransduced T cells expressed only background levels of IFNγ against all target cells. T cells expressing either FHVH33-CD828Z or FHVH33-CD8BBZ specifically recognized BCMA as shown by the high levels of IFNg released when the CAR-expressing T cells were cultured with the BCMA+ target cells BCMA-K562 an RPMI8226; in contrast, the CAR-expressing T cells produced much lower levels of IFNg when cultured with the BCMA-negative target cell lines Panc10.05, U251, 293GP, and the primary cells normal human bronchial epitheial cells (NHBE), human microvascular endotherlial cells (HMVEC), and primary human intestinal epithelial cells (InEpC). T cells alone made low levels of IFNγ for all T cell types evaluated. Compared to T cells expressing FHVH33-CD828Z, T cells expressing FHH33-CD8BBZ produced higher levels of IFNγ when cultured with the RPMI8226 cell line. The RPMI8226 cell line expresses low levels of BCMA. In many IFNγ ELISA experiments and other functional assays, T cells expressing FHVH33-CD8BBZ have shown a greater ability than T cells expressing FHVH33-CD828Z to be activated by RPMI8226 cells. These results suggest that compared to FHVH33-CD828Z, T cells expressing FHVH33-CD8BBZ have a greater ability to be activated by target cells expressing low levels of BCMA. Another point made by the data in **Table 2** is that CARs containing a 4-1BB moiety produce low levels of IFNγ when cultured alone or with BCMA-negative target cells. This production of IFNg with CAR T cells cultured in the absence of the CAR target antigen is a very consistent finding with 4-1BB CARs that target a variety of antigens including anti-CD19 CARs in clinical use and other anti-BCMA CARs.[51]

Table 2

	BCMA+		BCMA-negative						
Effector T cell	BCMA-K562	RPMI-8226	Panc10.05	U251	293GP	NHBE	HMVEC	InEpC	T-cells Only
Untransduced	179	406	76	41	92	54	54	33	25
FHVH33-828Z	38548	13855	37	24	40	43	25	18	14
FHVH33-8BBZ	70216	33465	306	360	238	423	610	500	367

All values are pg/mL of IFNγ

FHVH33-CD828Z-transduced T cells: 71% CAR expression

FHVH33-CD8BBZ-transduced T cells: 80% CAR expression

Table 3 shows ELISA results from an experiment in which effector T cells were cultured in the presence of BCMA protein at concentrations ranging from 150 ng/mL to 0 ng/mL. Effector T cells used in this experient were T cells from the same patient transduced with the SP6-CD828Z negative-control CAR, FHVH33-CD828Z, or FHVH33-CD8BBZ. Target cells used in this experiment were either BCMA-K562 cells or RPMI8226 cells or the BCMA-negative NGFR-K562 cells. The T cells and target cells were cultured together overnight, and IFNγ was measured in the culture supernatant by a standard ELISA assay. The presence of soluble BCMA protein did not block recognition of the BCMA+ target cells, and BCMA protein also did not cause nonspecific activation of the FHVH33-CD8BBZ T cells. All numbers are pg/mL IFNγ.

Table 3

BCMA-K562

Effector T cells	150ng/mL	50ng/mL	25ng/mL	0ng/mL
SP6-CD828Z	169	178	219	207
FHVH33-CD828Z	34821	38582	37225	35576
FHVH33-CD8BBZ	70008	63175	61903	60361

RPMI-8226

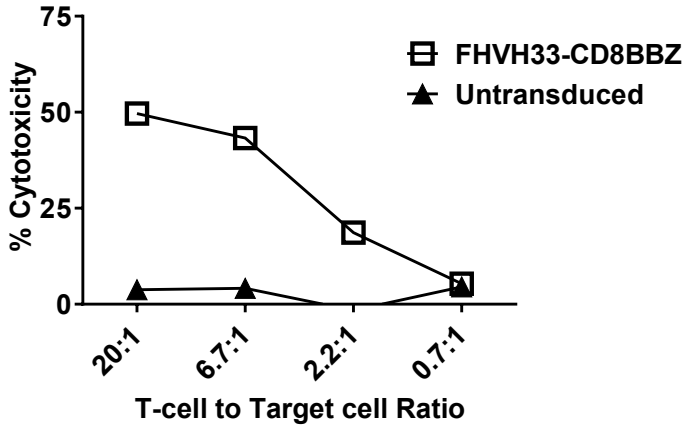
Effector T cells	150ng/mL	50ng/mL	25ng/mL	0ng/mL
SP6-CD828Z	247	259	210	253
FHVH33-CD828Z	14870	17680	16949	17583
FHVH33-CD8BBZ	30838	34304	30650	31937

T-cells Only

Effector T cells	150ng/mL	50ng/mL	25ng/mL	0ng/mL
SP6-CD828Z	115	116	136	121
FHVH33-CD828Z	24	18	21	22
FHVH33-CD8BBZ	117	114	125	122

Figure 7 presents the results of a 4 hour cytotoxicity assay in which RPMI8226 cells were used as target cells for either untransduced T cells or T cells transduced with FHVH33-CD8BBZ. The graph gives specific cytotoxicity of BCMA+ RPMI8226 cells relative to the negative-control BCMA-negative CCRF-CEM cell line. T cells expressing FHVH33-CD8BBZ were able to cause specific cytotoxity of the BCMA+ cell line.

Figure 7



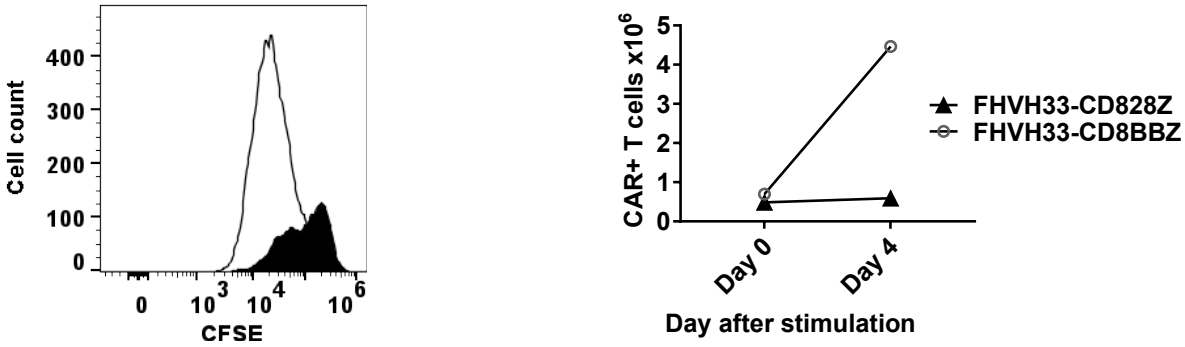
Anti-BCMA-CAR-transduced T cells also proliferated in a BCMA-specific manner.

Figure 8shows a carboxyfluorescein diacetate,succinimidyl ester (CFSE) proliferation assay in which anti-BCMA-CAR-transduced T cells were cultured for 4 days with either BCMA-K562

cells or BCMA-negative NGFR-K562 cells. CFSE was diluted to a greater degree, indicating more proliferation, when the T cells were cultured with BCMA-K562 target cells (open histogram in **Figure 8**) compared to when anti-BCMA-CAR T cells were cultured with BCMA-negative NGFR-K562 target cells (solid histogram in **Figure 8**). Note that the solid histogram representing culture of FHVH33-CD8BBZ T cells with NGFR-K562 cells is smaller than the open histogram representing culture of FHVH33-CD8BBZ T cells with BCMA-K562 cells because fewer T cells that were cultured with NGFR-K562 were left alive at the end of the assay. The assay was conducted as described previously.[5]

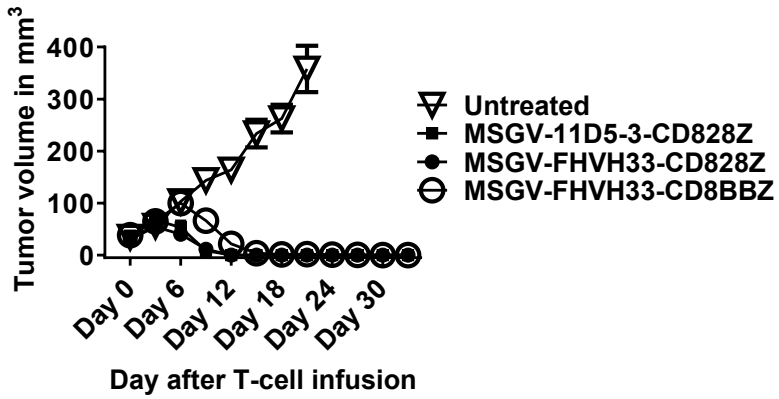
Figure 8 also includes a graph of CAR T-cell number at the beginning and at the end of the CFSE proliferation assay. The number of T cells transduced with FHVH33-CD8BBZ was compared to the number of T cells expressing FHVH33-CD828Z. In multiple experiments, we consistently found superior in vitro proliferation of T cells expressing FHVH33-CD8BBZ compared with T cells expressing FHVH33-CD828Z.

Figure 8



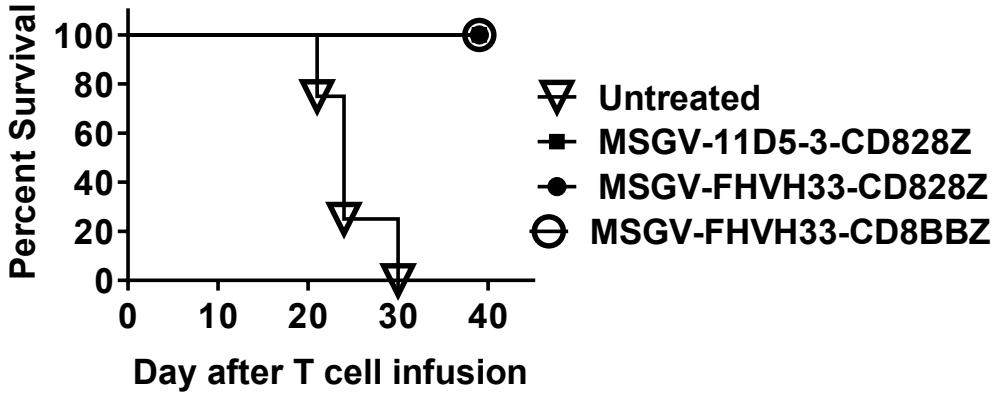
We established RPMI8226 human multiple myeloma cell line tumors in immunodeficient mice. We allowed sizable tumors to develop over 17 days then we treated the mice with a single intravenous infusion of anti-BCMA-CAR-transduced human T cells. After infusion of anti-BCMA-CAR T cells, dramatic regressions of all tumors occurred between day 6 and day 15 after the T cell infusion, and 100% of mice receiving anti-BCMA-CAR T cells were cured (**Figure 9** and **Figure 10**) The CARs used in this experiment included FHVH33-CD8BBZ, FHVH33-CD828Z, and the CAR used in our previous NCI anti-BCMA CAR trial, 11D5-3-CD828Z. In contrast, tumors continued to increase in size in all untreated mice. The mice receiving infusions of anti-BCMA-CAR-transduced T cells had no signs of toxicity during this experiment.

Figure 9



In the experiments depicted in **Figure 9** and **Figure 10**, anti-BCMA-CAR T cells were infused on Day 0, and no other treatments were administered. In the experiments reported in **Figure 9** and **Figure 10**, there were 5 mice in all groups.

Figure 10



1.2.9 Rationale for immunosuppressive chemotherapy and selection of lymphocyte-depleting chemotherapy regimen

We plan to administer a conditioning chemotherapy regimen of cyclophosphamide and fludarabine before infusions of anti-BCMA-expressing T cells because substantial evidence demonstrates an enhancement of the anti-malignancy activity of adoptively-transferred T cells when chemotherapy or radiotherapy are administered before the T cell infusions.[6,8,94] In mice, administering chemotherapy or radiotherapy prior to infusions of tumor-antigen-specific T cells dramatically enhanced the anti-tumor efficacy of the transferred T cells.[6,8,26,27,94,95] Administering chemotherapy or radiotherapy enhances adoptive T-cell therapy by multiple mechanisms including depletion of regulatory T cells and elevation of T-cell stimulating serum cytokines including interleukin-15 (IL-15) and interleukin-7 (IL-7), and possibly depletion of myeloid suppressor cells

and other mechanisms.[6,8,95,96] Removal of endogenous "cytokine sinks" by depleting endogenous T cells and natural killer cells caused serum levels of important T-cell stimulating cytokines such as IL-15 and IL-7 to increase, and increases in T-cell function and anti-tumor activity were dependent on IL-15 and IL-7.[6] Experiments in a murine xenograft model showed that regulatory T cells could impair the anti-tumor efficacy of anti-CD19 CAR T cells.[97] Myeloid suppressor cells have been shown to inhibit anti-tumor responses.[96] Experiments with a syngeneic murine model showed that lymphocyte-depleting total body irradiation (TBI) administered prior to infusions of anti-CD19-CAR-transduced T cells was required for the T cells to cure lymphoma.[7] In these experiments, some mice received TBI, and other mice did not receive TBI. All mice were then challenged with lymphoma and treated with syngeneic anti-CD19-CAR T cells. Mice receiving TBI had a 100% cure rate and mice not receiving TBI had a 0% cure rate.[7]

Strong suggestive evidence of enhancement of the activity of adoptively-transferred T cells has been generated in humans.[31,98,99] Very few clinical responses have occurred and very little evidence of in vivo activity has been generated in clinical trials of anti-CD19-CAR T cells administered without lymphocyte-depleting chemotherapy.[31,34] In contrast, many regressions and evidence of long-term B-cell depletion have occurred in clinical trials in which patients received anti-CD19-CAR T cells after lymphocyte-depleting chemotherapy.[3,4,33,52] The chemotherapy regimen that best increases the anti-malignancy efficacy of CAR-expressing T cells is not known, but the most commonly used chemotherapy regimens that have been used in clinical trials and that convincingly demonstrate persistence and in vivo activity of adoptively transferred T cells have included cyclophosphamide and fludarabine.[3,4,32,33,98,99] Both cyclophosphamide and fludarabine are highly effective at depleting lymphocytes.[98,99] One well-characterized and commonly used regimen is the combination of 300 mg/m^2 of cyclophosphamide administered daily for 3 days and fludarabine 30 mg/m^2 administered daily for three days on the same days as the cyclophosphamide.[100] Multiple cycles of this regimen can be tolerated by heavily pretreated leukemia patients.[66,100]

1.2.10 Rationale for dose-escalation and starting dose

The clinical trial described in this protocol is planned as a dose escalation in which the number of anti-BCMA-CAR T cells administered to patients will be increased with sequential dose levels. The rationale for conducting a dose-escalation trial of a cellular therapy is based on two main sources of evidence. First, the anti-tumor efficacy of adoptively-transferred T cell treatments increases as the dose of T cells administered to mice increases.[101-103] Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DLIs).[104,105] The incidence of graft-versus-host disease, which is caused by T cells attacking allogeneic antigens on host tissues, increases as the dose of T cells administered in DLIs increases'.[104,105]

The starting CAR T-cell dose of 0.75x10^6 CAR$^+$ T cells/kg is based on experience in past CAR clinical trials. The 0.75x10^6 CAR$^+$ T cells/kg dose is considered a dose lower than that at which substantial toxicity has been observed. We believe starting at a lower dose is prudent given that this is the first trial of a heavy-chain-only CAR. The following clinical trials were used to guide the starting dose of this trial. Note that it was our goal to use a dose lower than the MTD of prior trials. NCI protocol 09-C-0082 (Kochenderfer et al Journal of Clinical Oncology, 2017), a trial of anti-CD19 CAR T cells had an MTD of 2x10^6 CAR$^+$ T cells/kg.[66] NCI protocol 14-C-0168, a trial

of anti-BCMA CAR T cells had an MTD of $9x10^6$ CAR+ T cells/kg.[106] A multicenter trial of anti-BCMA CAR T cells conducted by bluebird bio, Inc. determined that a flat dose of 450 $x10^6$ CAR^+ T cells was the optimal dose for the expansion phase of the study (unpublished results). The 450 x 10^6 flat dose of CAR T cells would be $4.5x10^6$ CAR^+ T cells/kg for a 100 kg patient. Because the CAR to be tested in the current trial and the CAR tested in the bluebird bio trial both contain a 4-1BB domain, we predict the activity of the CAR to be tested in the current trial, FHVH33-CD8BBZ will be most like the CAR in the bluebird trial.

1.2.11 Summary of risks and potential benefits

This clinical trial is being performed to evaluate a genetically-modified T-cell therapy for multiple myeloma, which is an almost always incurable disease.[13] Only patients with multiple myeloma who have persisting or relapsed myeloma despite at least 3 prior lines of therapy will be enrolled. The risks of the study fall into 4 general categories. First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release-type toxicities such as high fevers, hypotension and neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. These cytokine-release-type toxicities have been detected in other clinical trials of CAR T cells during the first 2 weeks after anti-CD19 CAR T cells or anti-BCMA T cells were infused.[4,106,107] In most cases, these toxicities have been transient with toxicities generally resolving within 2 days to 2 weeks. The third main category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected cross-reactivity of the anti-BCMA CAR with proteins other than BCMA in vivo. We have not observed organ damage in our completed study of anti-BCMA CAR T cells.[106] A potential toxicity caused by anti-BCMA CAR T cells damaging normal cells is hypogammaglobulinemia due to depletion of plasma cells and a subset of mature B cells. Hypogammaglobulinemia has been a complication for many patients on clinical trials of anti-CD19 CAR-expressing T cells and anti-BCMA T cells.[4,33] . Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[4] Finally, genotoxicity is a theoretical risk of any type of integrating gene therapy. To our knowledge, genotoxicity, such as occurrence of a replication-competent retroviruses or transformation of T cells caused by insertional mutagenesis has never occurred in a clinical trials of T-cell gene therapies.[35,36] The specific gene therapy vector backbone, MSGV1, proposed for use in this clinical trial has been used in hundreds of patients over the past 13+ years by our group and others.[4,25,108,109]

The potential benefits to subjects enrolling on this trial include the possibility that the anti-BCMA-CAR T cells can cause a significant anti-myeloma effect. Many patients enrolled on early trials of anti-CD19 CAR T cells and anti-BCMA CAR T cells obtained prolonged complete remissions of advanced malignancies[3,4,106,110], so there is a chance that recipients of anti-BCMA CAR T cells on this clinical trial of fully-human heavy-chain-only CARs could also derive a direct benefit from participation in this trial. Patients might also derive a benefit from knowing that they are contributing to the development of new cellular therapies for cancer. Aiding in the development of new therapies might help future patients.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

Note: if a patient meets an eligibility requirement as outlined below, and is enrolled on the protocol but then is found to no longer meet the eligibility requirement after enrollment but before the start of protocol treatment, the treatment will be aborted or delayed until eligibility criteria are met.

2.1.1 Inclusion Criteria

2.1.1.1 Multiple Myeloma criteria

- BCMA expression must be detected on malignant plasma cells from either bone marrow or a plasmacytoma by flow cytometry or immunohistochemistry. If patient has plasmacytomas, one plasmacytoma must be biopsied to demonstrate BCMA expression. A specific quantitative level of BCMA expression for eligibility is not specified, but patients with multiple myeloma cells that are negative for BCMA by flow cytometry and immunohistochemistry on either bone marrow biopsy or plasmacytoma biopsy will not be enrolled. These assays must be performed at the National Institutes of Health (NIH). It is not required that the specimen used for BCMA determination comes from a sample that was obtained after the patient's most recent treatment. If paraffin embedded unstained samples of bone marrow involved with MM or a plasmacytoma are available, these can be shipped to the NIH for BCMA staining, otherwise new biopsies will need to be performed for determination of BCMA expression.
- BCMA expression will need to be documented on the majority of malignant plasma cells by flow cytometry at the NIH at some time after the original anti-BCMA CAR T-cell infusion in all patients undergoing a second anti-BCMA CAR T-cell infusion.
- Patients must have received at least 3 different prior treatment regimens for multiple myeloma
- Must have prior exposure to an "IMiD" such as lenolidamide and a proteasome inhibitor
- Patients must have measurable MM as defined by at least one of the criteria below.
  - One or more of these abnormalities defines measurable multiple myeloma:
  - Serum M-protein greater or equal to 1.0 g/dL.
  - Urine M-protein greater or equal to 200 mg/24 h.
  - Serum free light chain (FLC) assay: involved FLC level greater or equal to 10 mg/dL (100 mg/L) provided serum FLC ratio is abnormal.
  - A biopsy-proven plasmacytoma at least 2.0 cm in largest dimension
  - Bone marrow core biopsy with 30% or more plasma cells

2.1.1.2 Other inclusion criteria

- Greater than or equal to 18 years of age and less than or equal to age 73.
- Able to understand and sign the Informed Consent Document.
- Clinical performance status of ECOG 0-2

- Patients of both sexes must be willing to practice birth control from the time of enrollment on this study and for four months after last day of receiving protocol treatment.
- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.)
- A patient with a negative blood PCR test for hepatitis B DNA test can be enrolled. If hepatitis B DNA (PCR) testing is not available, patients with a negative hepatitis B surface antigen and negative hepatitis B core antibody can be enrolled.
- Patients must be tested for the presence of Hepatitis C antigen by PCR and be HCV RNA negative in order to be eligible. Only if Hepatitis C PCR testing is not available in a timely manner, patients who are Hepatitis C antibody-negative can be enrolled.
- Absolute neutrophil count greater than or equal to 1000/mm^3 without the support of filgrastim or other growth factors within the previous 10 days.
- Platelet count greater than or equal to 55,000/mm^3 without transfusion support within the past 10 days.
- Hemoglobin greater than 8.0 g/dL.
- Less than 5% plasma cells in the peripheral blood leukocytes
- Serum ALT and AST less or equal to 2.5 times the upper limit of the institutional normal.
- Serum creatinine less than or equal to 1.5 mg/dL.
- Total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL.
- At least 14 days must have elapsed since any prior systemic therapy at the time the patient starts the cyclophosphamide and fludarabine conditioning regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).
- Because this protocol requires collection of autologous blood cells by leukapheresis in order to prepare anti-BCMA-CAR T cells, systemic anti-myeloma therapy including systemic corticosteroid steroid therapy of greater than 5 mg/day of prednisone or equivalent dose of another corticosteroid are not allowed within 2 weeks prior to the required leukapheresis.
- Normal cardiac ejection fraction (greater than or equal to 50% by echocardiography) and no evidence of hemodynamically significant pericardial effusion as determined by an echocardiogram.
- For patients with past participation in gene-therapy, cryopreserved PBMC that have not been genetically-engineered must be available.

2.1.2 Exclusion criteria

- Patients on any anticoagulants except aspirin.
- Patients that require urgent therapy due to tumor mass effects or spinal cord compression.

- Patients that have active hemolytic anemia.
- Patients with second malignancies in addition to multiple myeloma are not eligible if the second malignancy has required treatment within the past 3 years or is not in complete remission. There are two exceptions to this criterion: successfully treated non-metastatic basal cell or squamous cell skin carcinoma.
- Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant. Women of child bearing potential cannot have a positive pregnancy test. Women of child-bearing potential are defined as all women except women who are post-menopausal or who have had a hysterectomy. Postmenopausal will be defined as women over the age of 55 who have not had a menstrual period in at least 1 year.
- Active systemic infections (defined as infections causing fevers or requiring anti-microbial treatment), active coagulation disorders or other major uncontrolled medical illnesses of the cardiovascular, respiratory, endocrine, renal, gastrointestinal, genitourinary, neurologic, or immune system, history of myocardial infarction, active cardiac arrhythmias including active atrial fibrillation, history of any arrhythmias other than sinus tachycardia or atrial fibrillation, currently taking any anti-arrythmic or congestive heart failure medications, active obstructive or restrictive pulmonary disease.
- Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
- Systemic corticosteroid steroid therapy of greater than 5 mg/day of prednisone or equivalent dose of another corticosteroid (prednisone, dexamethasone, etc) is not allowed within 2 weeks prior to either the required leukapheresis or within 2 weeks prior to CAR T-cell infusion (and at any time after the CAR T cell infusion).
- History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- Patient unwilling to undergo intensive care unit treatment including mechanical ventilation, cardiopulmonary resuscitation, vasoactive drugs, and hemodialysis.
- History of allogeneic stem cell transplantation
- Patients with current spinal cord compression (without intradural myeloma involvement).
- Patients who have a history (or current evidence) of cerebrospinal fluid multiple myeloma, or intra-dural central nervous system masses.
- Patients with active autoimmune skin diseases such as psoriasis or other active autoimmune diseases such as rheumatoid arthritis.
- Patients must not have required supplemental oxygen within the past month unless it was for a resolved infection.
- Patient must not have received genetically modified cells except on prior NCI gene therapy protocols.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on the NIH websites and on NIH social media platforms. Participants will be recruited from the current patient population at NIH, and local community physicians.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed after a consent for screening has been signed

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01C0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

The following assessments must be completed within **30 days** prior to starting the chemotherapy conditioning regimen unless otherwise noted (if not, then the evaluation must be repeated):

- Complete history and physical examination, including: weight, vital signs, ECOG
- Confirmation of diagnosis of MM by the NCI Laboratory of Pathology and confirmation of BCMA expression on malignant plasma cells from either bone marrow or a plasmacytoma by flow cytometry or immunohistochemistry. The sample used for this BCMA expression analysis can come from any time prior to enrollment on the protocol.
- Bone marrow aspirate and biopsy specifically ask for BCMA immunohistochemistry staining of the bone marrow biopsy. Order cytogenetics with interphase FISH (pretreatment aspirate only) and flow cytometry on the bone marrow aspirate. Specifically request 2 separate bone holes for bone marrow aspiration. The aspirate for hemepath (0.5 mL) and flow (2-2.5 mL) should be done from 1 bone hole and the aspirate for research (2-2.5 mL) should be done from a second bone hole followed by an aspirate for cytogenetics (1-2 mL) from the second bone hole. Note: Only bone marrow aspirates done before CAR T-cell treatment and 2 weeks after CAR T-cell infusion require aspirates through 2 bone holes. All other aspirates only need to be done through 1 bone hole.

  The bone marrow biopsy must take place at some time after the patient's most recent myeloma treatment. If the patient agrees to future use of specimens when screening bone marrow is obtained, reserve one tube of bone marrow aspirate to be sent to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Bone marrow aspirate cells and supernatant will be cryopreserved. As many vials as possible with 10 million cells or less will be cryopreserved, and 2 vials of supernatant with 0.5 to 1 mL each will be cryopreserved. A bone marrow biopsy must be performed within 24 days of the start of the protocol-required conditioning chemotherapy. If this screening bone marrow aspirate and biopsy is performed within 24 days of the start of the conditioning chemotherapy, a second baseline bone marrow biopsy does not need to be performed.
- All patients must have flow cytometry staining of bone marrow or plasmacytoma cells for BCMA by Dr. Stetler-Stevenson's lab (NCI Laboratory of Pathology).
- EKG
- MRI of spine and pelvis (only if clinically indicated in patients with back pain or pelvic pain or a history of plasmacytomas of spine or pelvic)

- MRI of the brain
- If necessary to assess disease, obtain a skeletal X-ray survey
- PET scan of torso and/or extremities (only if needed to evaluate disease)
- CT scans of areas with possible lesions/plasmacytomas This may include areas such as the head, neck, chest, abdomen and pelvis. (only if needed to evaluate disease)
- Donor venous assessment
- DNA (PCR) for Hepatitis B and C; antibody testing for HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR), only if DNA testing is not available, order Hepatitis C antibody, Hepatitis B core antibody, and hepatitis B surface antigen.
- Echocardiogram
- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid)
- CBC with differential and platelet count
- Serum immunofixation electrophoresis
- Serum immunoglobulin free light chains
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening
- TSH (T4 and T3)
- Serum Cortisol
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

2.3 Participant Registration and Status Update Procedures

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

2.4 Treatment Assignment Procedures for the Registartion Purposes Only:

Cohorts

Number	Name	Description
1	Cohort 1	Subjects enrolled to determine the MTD or maximum feasible dose
2	Cohort 2	Subjects enrolled after the MTD or maximum feasible dose has been identified

Arms

Number	Name	Description
1	Conditioning chemotherapy plus CAR T-cells dose escalation	Patients will receive escalating doses (up to 5 planned) of CAR+ T cells infused on day 0 + Cyclophosphamide: 300 mg/m^2 IV infusion over 30 minutes on days -5, -4 and -3 + Fludarabine: 30 mg/m^2 IV infusion over 30 minutes administered immediately following the cyclophosphamide on days -5, -4,and -3
2	Conditioning chemotherapy plus CAR T-cells expansion phase	6.0x10^6 cells/kg dose (maximum feasible dose) of CAR T Cells + Cyclophosphamide: 300 mg/m^2 IV infusion over 30 minutes on days -5, -4 and -3 + Fludarabine: 30 mg/m^2 IV infusion over 30 minutes administered immediately following the cyclophosphamide on days -5, -4,and -3

Arm Assignment

Patients in Cohort 1 will be directly assigned to Arm 1.

Patients in Cohort 2 will be directly assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

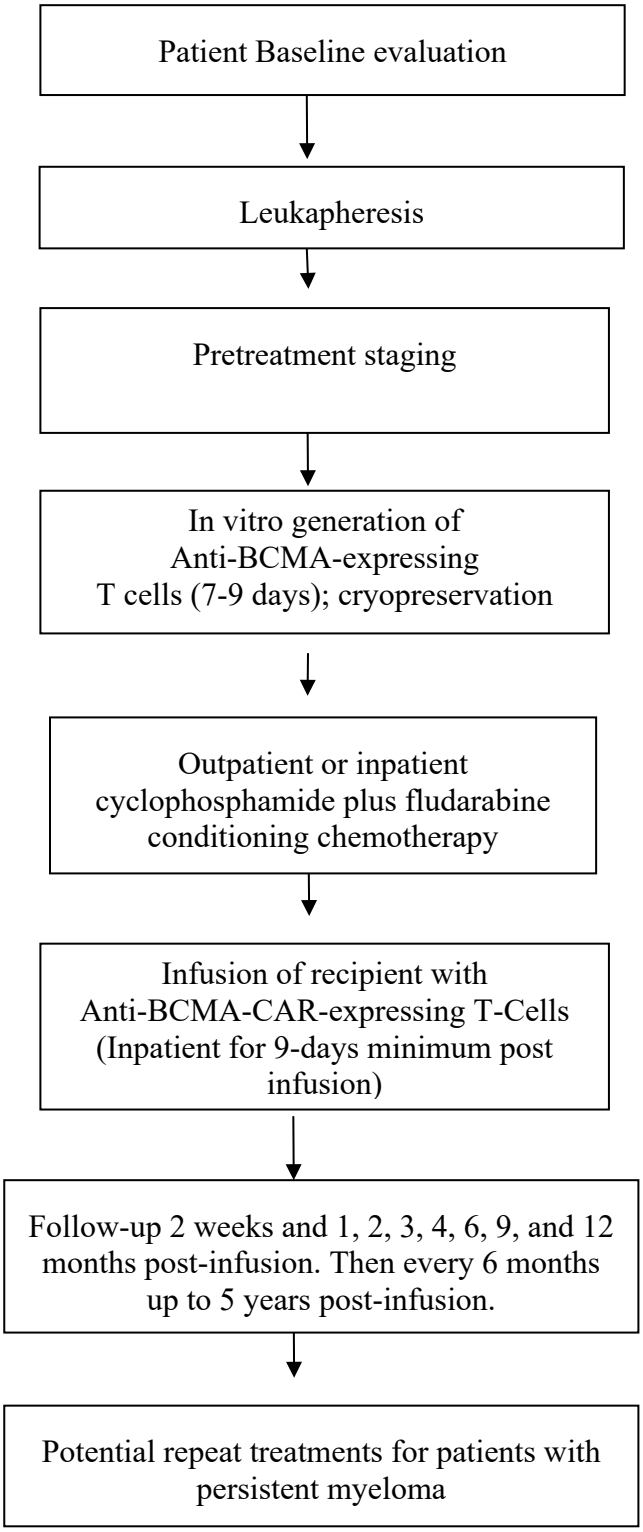
3.1.1 General Study Plan

This protocol is a phase I dose-escalation study of autologous T cells that are genetically modified to express an anti-BCMA CAR.

The protocol will enroll patients with multiple myeloma previously treated with at least 3 prior lines of therapy. Patients will be evaluated for general health, and multiple myeloma staging will be carried out. An assessment of BCMA expression will be an important part of the eligibility screening. Patients enrolled on the study will undergo leukapheresis, and anti-BCMA-CAR-expressing T cells will be generated by transducing the patient's T cells with a gammaretrovirus encoding the anti-BCMA CAR. Patients will receive a conditioning chemotherapy regimen of cyclophosphamide 300 mg/m^2 daily for 3 days and fludarabine 30 mg/m^2 IV daily for 3 days on the same days. This is an extensively-used chemotherapy regimen that can be easily administered on an outpatient basis. Two days after the end of the conditioning chemotherapy, patients will receive a single infusion of anti-BCMA-CAR-expressing T cells. A minimum 9-day hospitalization will be required after the cell infusion to monitor closely for acute toxicities. It is expected that some patients will need to be hospitalized for up to 30 days to allow all toxicities to resolve to the point that discharge from the hospital is prudent. Patients are required to stay within 60 minutes driving time from the Clinical Center until day 14 after the CAR T-cell infusion. Patients will then be evaluated for toxicity and multiple myeloma will be staged 2 weeks and 1, 2, 3, 4, 6, 9, and 12 months after the infusion. They will be followed every 6 months afterwards for at least 5 years.

A small number of subjects may be eligible for re-enrollment if a patient is removed from the protocol before completing protocol treatment; or the patient is going to receive a second treatment. These patients would be required to meet all eligibility criteria at the time of re-enrollment. Patients will be assigned a new sequential study number for the reenrollment study period. Any cryopreserved anti-BCMA CAR T cells produced from a patient who was removed from the study can be used to treat that patient after re-enrollment. We do not anticipate changes in the risk profile for the initial versus re-enrollment

3.1.2 Protocol Schema



3.1.3 Dose Limiting Toxicity-the dose limiting toxicity assessment period is 28 days.

DLTs are defined as follows (Note that DLTs will not occur in patients who do not receive CAR T-cell infusions but do receive the conditioning chemotherapy):

- Grade 3 toxicities possibly or probably or definitely related to the anti-BCMA CAR T cells and lasting more than 9 days
- Grade 4 toxicities possibly or probably or definitely related to the anti-BCMA CAR T cells.

The following specific toxicities will not be DLTs:

- Neutropenia (ANC<500/μL) lasting 10 days or less is not a DLT
- Neutropenia with an ANC>500/mL is not a DLT
- Anemia (Hgb<8 g/dL) lasting 10 days or less is not a DLT
- Anemia with Hgb greater than or equal to 8 g/dL is not a DLT
- Transfusion-dependent thrombocytopenia lasting 28 days or less is not a DLT
- Thrombocytopenia that is not transfusion-dependent is not a DLT
- Hypotension requiring continuous treatment with vasopressors for 72 hours or less is not a DLT.
- Fever is not a DLT.
- All cytopenias except neutropenia, anemia, and thrombocytopenia are not DLTs
- Asymtomatic electrolyte disturbances regardless of grade are not DLTs
- Prolonged QT interval as long as ventricular arrhythmias do not occur is not a DLT
- Grade 1, 2, or 3 creatine kinase elevation is not a DLT
- Infections controlled by antibiotics are not DLTs

3.1.4 Dose Escalation

The trial will be a dose-escalation with 5 dose levels based on the patient's **actual** bodyweight.

Dose Escalation Schedule	
Dose Level	Dose of IND Agent
Level -1	$0.4x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 1	$0.75x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 2	$1.5x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 3	$3.0x10^6$ CAR+ T cells per kg of recipient bodyweight

Dose Escalation Schedule	
Dose Level	Dose of IND Agent
Level 4	$6.0x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 5	$12.0x10^6$ CAR+ T cells per kg of recipient bodyweight

A minimum of 3 patients will be enrolled at each dose level. There will be a minimum of 9 days between the CAR T-cell infusion of a patient and the start of the conditioning chemotherapy regimen for the next patient. This will cause a 14 day delay between sequential CAR T-cell infusions.

If sufficient cells cannot be grown to meet the criteria for the assigned dose level but sufficient cells can be grown to meet the requirements of one dose level lower than the assigned dose level, the patient will be enrolled in the appropriate dose level for the number of cells infused. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at this level as described in the dose-escalation scheme below. Accrual will be halted at the higher level until the dose level at the lower level is complete as described below. If sufficient cells cannot be produced to infuse the number of cells called for by one dose level lower than the called for dose level, the treatment will be aborted. A second attempt will be made to prepare cells for the patient if the patient agrees and if the patient still meets all eligibility criteria.

Should none of the first 3 patients treated on a dose level experience a DLT, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the third patient. Should 1 of 3 patients experience a dose limiting toxicity at a particular dose level, three more patients would be treated at that dose level. If 1/6 patients have a DLT at a particular dose level, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the 6^{th} patient. If a level with 2 or more DLTs in 3-6 patients has been identified, 3 additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the MTD.

If 2 of 3 patients on Dose level 1 experience DLTs, accrual will proceed on dose level -1.

The MTD is the dose at which a maximum of 1 of 6 patients has a DLT. After a MTD is defined, additional patients can be treated at the MTD. Up to 12 total additional recipients can be treated after an MTD is established to more completely characterize toxicity of the MTD. If cell growth limitations preclude administration of the MTD, the patient will receive as many cells as possible up to the MTD. If it proves to be technically impossible or impractical to achieve the higher dose levels due to cell production constraints and a MTD has not been reached, the highest achievable dose level will be declared the maximum feasible dose, and up to 12 additional recipients will be treated with the maximum feasible cell dose.

The maximum feasible dose has been determined to be $6x10^6$ CAR^+ cells/kg based on technical difficulty preparing larger numbers of CAR^+ T cells and lack of establishment of a MTD after treating at least 3 patients on all dose levels.

Dose escalation will follow the rules outlined in the Table below:

Number of Patients with DLTat a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter up to 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at the highest dose or 1 level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

3.2 DOSE MODIFICATIONS/DELAY

Other Toxicity:

- Patients may be removed from further treatment if they have active infections defined as infections causing fevers or infections requiring anti-microbial therapy that arise while patients are on-study but before the CAR T-cell infusion; however such patients are eligible for treatment if they meet all eligibility criteria after the infection resolves.
- If a patient experiences a grade 3 or greater toxicity (with the exception of cytopenias including neutropenia, lymphopenia, anemia, or thrombocytopenia) while on-study before the CAR T-cell infusion, the CAR T-cell infusion must be delayed until the toxicity improves to a grade 2 or less. Exceptions to this would be if the grade 3 toxicity was present at baseline or related to progressing disease.

3.3 DRUG ADMINISTRATION

3.3.1 Leukapheresis

The patient will undergo a 10-20 liter leukapheresis (generally, 15 liters will be processed to target a yield of 6-10 $x10^9$ lymphocytes) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. The procedure requires dual venous access, and takes approximately 3-4 hours to complete. A central line will be placed if peripheral venous access is not sufficient.

3.3.2 Anti-BCMA-CAR-expressing T-cell preparation

After cells are obtained by apheresis, further cell processing to generate anti-BCMA CAR-expressing T cells will occur in the DTM according to standard operating procedures (SOPs). Either freshly-collected cells or cryopreserved cells can be used to initiate the cell-preparation process. In addition as stated in eligibility criteria, cryopreserved cells stored continuously in the Center for Cellular Engineering can be used to prepare autologous clinical cell products on other NCI cell therapy protocols, if the future cell therapy is conducted under a different NCI protocol. Peripheral blood mononuclear cells will be isolated. Sufficient cells for 2 complete cell productions will be retained in the Department of Transfusion Medicine; the excess cells will be sent to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755for cryopreservation at 100 to 200 million PBMC per vial. These cells will be used in research. The anti-CD3 monoclonal antibody OKT3 will be used to stimulate T-cell proliferation. Two days after the start of the T-cell cultures, the cells will be transduced by exposing them to a supernatant containing replication-incompetent gammaretroviruses encoding the anti-BCMA CAR. The cells will continue to proliferate in culture. The total cell production process will take 7 to 9 days before cells are cryopreserved. Ten vials of the infused cells will be cryopreserved and stored in the Surgery Branch Cell Production Facility SB-CPF. Each vial will contain 10-20 million cells.

Before infusion, the percentage of T cells expressing the anti-BCMA CAR will be determined by flow cytometry, and this percentage of BCMA+ T cells will be used in calculating the total number of cells to be infused to meet the dose requirements of the dose-escalation plan described in **3.1.4** In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent retroviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. When a patient is no longer eligible for retreatment on this protocol due to meeting any of the off-study criteria listed in section **3.8.2**, any remaining cryopreserved pretreatment PBMC collected on this protocol will be transferred from the Department of Transfusion Medicine to the Principal Investigator of this protocol for storage in the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755 and possible use in research.

Note: All cells sent to the Surgery Branch Cell Production facility will be transferred to NCI Frederick Biorepository.

3.3.3 Conditioning chemotherapy and anti-BCMA CAR T-cell administration-chemotherapy administration can be either inpatient or outpatient

3.3.3.1 Overall summary of the treatment plan

Drug	**Dose**	**Days**
Cyclophosphamide	300 mg/m^2 IV infusion over 30 minutes	Daily x 3 doses on days -5, -4, -3
Fludarabine	30 mg/m^2 IV infusion over 30 minutes administered immediately following the	Daily x 3 doses on days -5, -4, -3

Drug	Dose	Days
	cyclophosphamide on day -5, -4, -3	
Anti-BCMA CAR T cells	Variable.	One time dose on day 0

3.3.3.2 Detailed treatment plan

Day -5, -4, and -3: Patients will receive pre-hydration with 1000 mL 0.9% sodium chloride I.V. over 1 to 3 hours.

Patients will receive anti-emetics following NIH Clinical Center guidelines, but **dexamethasone will not be administered**. One suggested regimen is ondansetron 16 to 24 mg orally on days -5, -4, and -3 before chemotherapy (I.V. ondansetron can be substituted). Patients should be provided with anti-emetics such as lorazepam and prochlorperazine to use at home.

On days -5, -4, and -3, cyclophosphamide at a dose of 300 mg/m^2 I.V. will be diluted in 100 mL 5% dextrose solution and infused over 30 minutes. After the cyclophosphamide on days -5, -4, and -3, patients will receive 30 mg/m^2 I.V. fludarabine in 100 mL 0.9% sodium chloride over 30 minutes. **Note: in patients with an estimated creatinine clearance of 30 - 70 mL/minute/1.73m^2, the daily dose of fludarabine will be reduced by 20%.** Creatinine clearance will be estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

Following the fludarabine infusion, patients will receive 1000 mL 0.9% sodium chloride I.V. over 1-2 hours. Furosemide will be given if needed.

Days -2 and -1: No interventions except as needed for general supportive care such as anti-emetics. To minimize bladder toxicity, patients should increase normal oral fluid intake to at least 2 L/day.

Day 0: Anti-BCMA CAR T cells will be administered. Premedication for the cell infusion will be given approximately 30 minutes prior to the infusion. The premedications are acetaminophen 650 mg orally and diphenhydramine 12.5 mg IV. Cells are delivered to the patient care unit from the Department of Transfusion Medicine. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and identification of the product and documentation of administration are entered in the patient's chart as is done for blood banking protocols. The cells are to be infused intravenously over 20 to 30 minutes via a central line with non-filtered tubing through an **INFUSION PUMP**, gently agitating the bag during infusion to prevent cell clumping. Cells may arrive on the unit in a syringe instead of a bag. In this case, the cells can be administered to the patient by pushing the cells through a free flowing normal saline line per nursing SOP, but still must be infused over 20-30 minutes. Details of the infusion procedure are included in **Appendix D** (Note: The cell infusion may be delayed up to a day per the PI's discretion)

Days 0 to 9: Mandatory hospitalization for observation and treatment as necessary. Note: hospitalization for CAR T-cell patients is routinely extended; extension of hospitalization beyond the required 9 days is anticipated for CAR T cell toxicity management. In addition, patients are

required to stay within 60 minutes driving time from the Clinical Center until day 14 after the CAR T-cell infusion.

Guidelines for dealing with toxicities that often occur after CAR T cell infusions including hypotension, fever and tachycardia are given in **15.3**.

A CBC with differential will be obtained daily. If the absolute neutrophil count becomes less than 500/microliter, filgrastim will be initiated at a dose of 300 micrograms daily subcutaneously for patients under 70 kg in weight and at a dose of 480 micrograms daily for patients over 70 kg in weight. Filgrastim will be given daily and then discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.

3.3.4 Potential repeat treatment

Patients obtaining any response except progressive disease are potentially eligible for a repeat treatment consisting of conditioning chemotherapy followed by an infusion of anti-BCMA CAR T cells. Retreatment will be at least 2 months after the original treatment. The dose of anti-BCMA CAR T cells administered during repeat treatments will be the dose level currently enrolling patients receiving initial CAR T-cell infusions. The repeat treatment will include the same conditioning chemotherapy as the initial treatment.

To be eligible for a repeat treatment, patients must have not experienced a DLT with their first treatment. Patients must also meet the same eligibility requirements listed in Section **2.1**. BCMA expression must be documented after the first anti-BCMA CAR infusion and before the second BCMA CAR T-cell infusion. The patients must undergo screening evaluation as listed in Section **2.2** except infectious disease serology screening is not required to be repeated unless clinically indicated. Follow-up testing for retreatment will be the same as for the first treatment. The patient may be re-enrolled on the the study as a new patient to allow this re-treatment and these patients will be considered in the total sample size for this study. All patients receiving a second treatment will be identified as patients receiving repeat treatments in any reports of results of this trial. A maximum of 2 total treatments can be administered to any one patient, and at least 2 months must elapse between the first treatment and the second treatment.

Second treatments including DLTs will not affect the dose escalation plan described in section **3.1.4**. DLTs that occur in patients receiving a repeat treatment will not affect the dose escalation of patients receiving an initial treatment. However, excessive DLTs among re-treatments will result in a discontinuation of this practice. Specifically, if 2 of the first 3 patients re-treated, 3 or more of the first 6 re-treated patients, 4 or more out of the first 9 re-treated patients, or greater than 1/3 of the total patients receiving a repeat treatment experiences a DLT during repeat treatment, then repeat treatments will be discontinued altogether.

3.4 PROTOCOL EVALUATION

3.4.1 Baseline evaluations and interventions

Bone marrow aspirate and biopsy must be performed within 24 days of the start of the conditioning chemotherapy. If the screening bone marrow aspirate and biopsy were done within 24 days of the start of conditioning chemotherapy, the baseline bone marrow aspirate and biopsy should not be done unless clinically indicated. Specifically ask for BCMA, CD3, CD8, and CD4 immunohistochemistry staining of the bone marrow biopsy. Order cytogenetics with interphase FISH (pretreatment only) and flow cytometry on the bone marrow aspirate. Specifically request 2

separate bone holes for bone marrow aspiration. The aspirate for hemepath (0.5 mL) and flow (2-2.5 mL) should be done from 1 bone hole and the aspirate for research (2-2.5 mL) should be done from a second bone hole followed by an aspirate for cytogenetics (1-2 mL) from the second bone hole. Note: Only bone marrow aspirates done before CAR T-cell treatment and 2 weeks after infusion require aspirates throught 2 bone holes. All other bone marrow aspirates only need to be done through 1 bone hole. For all bone marrow aspirates, bone marrow supernatant must be collected and frozen in the same manner as serum is collected and frozen.

BCMA immunohistochemical staining was performed using Ventana Ultra automated staining system (Ventana, Tucson, AZ) according to manufacturer's directions. Incubation in mouseanti-BCMA primary antibody (Santa Cruz Biotechnology clone D6) was followed by anti-mouse secondary antibody incubation. Flow cytometry on bone marrow aspirates was performed by using standard methods and the Santa Cruz Biotechnology clone D6 antibody that is specific for BCMA.

The following tests must be completed within 14 days prior to the start of the conditioning chemotherapy regimen:

- Patients must have a central venous access before the time of cell infusion. This might require placement of a non-valved P.I.C.C line or other device.
- Physical exam with vital signs and oxygen saturation
- PET-CT scan in all patients
- CT scans of areas with possible lesions/plasmacytomas. This may include areas such as the head, neck, chest, abdomen and pelvis. (If needed to stage MM) Spine MRI if needed to stage MM
- Skeletal survey. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
- Serum immunofixation electrophoresis
- 250 microgram cosyntropin stimulation test if suspicious for adrenal insufficiency based on low serum sodium or high serum potassium or hypotension or a history of adrenal insufficiency or low serum cortisol or other clinical indications (This may be performed after start of chemotherapy as long it is performed before the cell infusion)
- 24-hour urine collection with immunofixation electrophoresis timed urine
- 24-hour urine collection for urine protein
- Serum immunoglobulin free light chains
- Anti CMV antibody titer, HSV serology, and EBV panel, T cruzi serology, toxoplasmosis serology (Note: patients who are known to be positive for any of the above do not need to be retested; may be performed within 3 months of chemotherapy start date)
- Blood will be collected for research purposes. Draw 12 CPT tubes (8 mL each of blood will be collected prior to initiation of the conditioning chemotherapy regimen. This is a total of 96 mL of blood. Some of this blood will be used for immunology assays and some will be used for RCR assays. This blood can be collected on different days as long as a

total of 12 CPT tubes are collected prior to cell initiation of the chemotherapy. Send to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.

- In addition to the CPT tubes, draw 16 mL of blood to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 3 days prior to the start of the chemotherapy. Send to the Biospecimen Processing Core (BPC) lab; For sample pick-up, page 102-11964.

The following tests must be completed within 7 days of the start of the conditioning chemotherapy regimen:

- TBNK
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK (creatine kinase), Uric Acid (to be repeated on the first day of the chemotherapy at the discretion of the PI)
- β2-microglobulin
- ABO typing
- Ionized calcium
- CBC with differential and platelet count
- PT/PTT
- Fibrinogen
- Urinalysis; if results are abnormal, send for urine culture
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- C-reactive protein (CRP)

3.4.2 Studies to be performed on Day 0 and during the mandatory 9-day inpatient admission after cell infusion

- Vital signs including pulse oximetry will be monitored q1h x 4 hours (+/- 15 minutes) after completion of the CAR T cell infusion and approximately every 4 hours (+/-15 minutes) otherwise unless otherwise clinically indicated.
- Daily physical exam
- CBC twice a day from day 0 until day 9 with differential daily. After day 9 do a CBC with differential daily until discharge. (In the case of a later day infusion or early discharge, this may be only once a day.)
- TBNK on the day of CAR T-cell infusion (day 0), day 7 and day 14 after infusion
- Chemistries twice a day starting from day 0 to day 9. After day 9 do chemistries once a day until discharge: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total

(Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Uric Acid, creatine kinase. (In the case of a later day infusion or early discharge, this may be only once a day.)

- PT/PTT and fibrinogen daily while hospitalized
- **Day 0 Research Blood:** 1 SST tube will be drawn on the morning of CAR cell infusion prior to infusion of CAR T cells. Send to the BPC lab; for sample pick-up, page 102-11964.
- **Post-infusion Research blood:** Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the day of CAR T-cell infusion and lasting up to 20 days after infusion of anti-BCMA-CAR-transduced T cells, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Send CPT tubes to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Send SST tubes to the BPC lab; for sample pick-up, page 102-11964.
- **Additional Post-infusion research blood:** 1 SST tube will be drawn on the first Sunday after CAR T-cell infusion. This tube will be stored refrigerated on the nursing unit and processed first thing Monday morning at the latest. Send to the BPC lab; For sample pick-up, page 102-11964.

Note regarding research blood collection: If any of the above time points fall on federal holiday then, we may collect research blood on the next day.

3.4.3 Post-infusion outpatient evaluation

After completion of therapy the patient will be followed for potential complications related to anti-BCMA–CAR T-cell infusion. The patient will be seen at the NIH in follow-up to evaluate disease status and late problems related to anti-BCMA –CAR T-cell infusion at days +14 (+/- 1 day), +30 (+/- 5 days), +60(+/- 7 days), +90(+/- 7 days), and +120(+/- 7 days); and at 180(+/- 14 days), 270 (+/- 14 days), and 365 (+/- 30 days) months after anti-BCMA –CAR T-cell infusion. After 12 months, the patient will be seen every 6 months (+/- 30 days) up to five years. All of these time points have allowable ranges (see Table in section **3.5**). At these times patients will have the following tests performed to determine clinical response:

- 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Send to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.
- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Send to the BPC lab; For sample pick-up, page 102-11964.

  Note: **after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits**.
- PET scans will be performed if needed to stage disease as described in section **3.5.**
- CT scans of areas with lesions/plasmacytomas starting 1 month after infusion. This may include areas such as the head, neck, chest, abdomen and pelvis. (Only if a plasmacytoma/lesion was detected before treatment)

- MRI of spine (Only if a plasmacytoma/lesion was detected before treatment)
- Physical exam with vital signs and oxygen saturation
- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid
- PT/PTT, fibrinogen
- TBNK
- Ionized calcium
- CBC with differential
- C-reactive protein (CRP)
- Blood for serum β2-microglobulin
- Blood for immunoglobulin free light chains
- Blood for serum immunofixation electrophoresis
- 24-hour urine collection with immunofixation electrophoresis timed urine (can be omitted if not needed for staging after day 30).
- 24-hour urine collection with protein timed urine (can be omitted if not needed for staging after day 30).
- Urinalysis: if results are abnormal, send for urine culture
- At the 2 week follow-up, bone marrow aspirate and biopsy must be performed. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. BCMA staining must be requested for the flow cytometry. BCMA immunohistochemistry should also be requested on the bone marrow biopsy.Specifically ask for <u>BCMA, CD3, CD8, and CD4 immunohistochemistry</u> staining of the bone marrow biopsy. Specifically request 2 separate bone holes for bone marrow aspiration. The aspirate for hemepath (0.5 mL) and flow (2-2.5 mL) should be done from 1 bone hole and the aspirate for research (2-2.5 mL) should be done from a second bone hole.
- At 2 week follow-up, a peripheral blood sample will be collected. Peripheral blood must be sent for flow cytometry analysis to the lab of Dr. Maryalice Stetler-Stevenson to evaluate for malignant plasma cells, the BCMA expression on any malignant plasma cells present and CAR positive T cells.
- At the 2 month follow-up, a bone marrow aspirate and biopsy will be collected. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. BCMA staining must be requested for the flow cytometry. BCMA immunohistochemistry should also be requested on the bone marrow biopsy. This may also be performed at day +30 if clinically indicated. Specifically ask for <u>BCMA, CD3, CD8, and CD4 immunohistochemistry</u> staining of the bone marrow biopsy. The aspirate for hemepath (0.5 mL), flow (2-2.5 mL) and research (2-2.5 mL) can be done from 1 bone hole.

- At the 6-month, 18-month, 30 -month, and 42-month follow-up appointments, a bone marrow aspirate and biopsy will be performed. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. BCMA staining must be requested for the flow cytometry. BCMA immunohistochemistry should also be requested on the bone marrow biopsy. Specifically ask for BCMA, CD3, CD8, and CD4 immunohistochemistry staining of the bone marrow biopsy. The aspirate for hemepath (0.5 mL), flow (2-2.5 mL) and research (2-2.5 mL) can be done from 1 bone hole.
- For each bone marrow aspirate performed, send one tube of bone marrow aspirate to Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Bone marrow cells should be cryopreserved and the liquid bone marrow supernatant should also be saved at -80 degrees. As many vials as possible with 10 million cells or less will be cryopreserved, and 2 vials of supernatant with 0.5 to 1 mL each will be cryopreserved.
- Gene-therapy-specific follow-up must be carried out as described in section **3.6**

3.5 STUDY CALENDAR

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Follow up							
				Day+14 (+- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 months after day 365(+/- 30 days) up to 5 years
Clinical Assessments											
History and PE	X	X	X	X	X	X	X	X	X	X	X
Vital signs, O2 saturation[o]	X	X	X	X	X	X	X	X	X	X	X
Height, weight	X[s]	X[s]									
Performance Score	X	X									
Laboratory Assessments											
CBC with differential	X	X	X	X	X	X	X	X	X	X	X
Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT, AST, T. Bilirubin, D. Bilirubin, LDH, Total Protein, CK, Uric Acid	X	X	X	X	X	X	X	X	X	X	X
Serum C-reactive peptide	X[h]	X[m]	X[m]	X	X	X	X	X	X	X	X
PT/PTT, Fibrinogen	X[h]		X	X	X	X	X	X	X	X	X
Ionized calcium	X[h]			X	X	X	X	X	X	X	X
Thyroid Stimulating Hormone, T3	X[K]										

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Follow up							
				Day+14 (+- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 months after day 365(+/- 30 days) up to 5 years
β2-HCG (serum or urine in women of childbearing potential)	X										
β2-microglobulin	X[h]			X	X	X	X	X	X	X	X
Anti-CMV, HSV, EBV, T.cruzi,toxoplasma	X[h,j]										
Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, West Nile, and syphilis (RPR)	X										
TBNK	X[h]	X	X	X	X	X	X	X	X	X	X
G6PD	X[K]										
Urinalysis (culture prn)	X[h]			X	X	X	X	X	X	X	X
24 hr urine with immunofixation electrophoresis timed urine and urine 24 hour protein	X[h]			X	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]
ABO typing	X[h]										
Serum immunoglobulin free light chains	X			X	X	X	X	X	X	X	X
Serum immunofixation electrophoresis	X			X	X	X	X	X	X	X	X

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Follow up							
				Day+14 (+- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 months after day 365(+/- 30 days) up to 5 years
Peripheral blood flow cytometry				X[t]							
Radiological Assessments											
Skeletal Survey	X										
CT scan of disease affected areas	X[u]			X[i,e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]
PET	X[h]				X[f]			X[f]	X[f]	X[f]	X[f]
Brain MRI	X										
Spine MRI [e]	X			X [e]	X [e]	X [e]	X [e]	X [e]	X [e]	X [e]	X [e]
Other Specific Assessments											
Serum Cortisol	X[k]										
250 microgram cosyntropin test [c]	X[h]										
EKG, echocardiogram	X										
RCR	X					X		X		X	X[g]
Central Venous catheter placement		X									
Venous Assessment	X										
Response Evaluation											
Bone marrow aspirate/biopsy [d, q]	X			X[l]	X			X			X[v]
Research Blood [n. p. r]	X [h]	X[n]	X	X	X	X	X	X	X	X	X

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Follow up							
				Day+14 (+- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 months after day 365(+/- 30 days) up to 5 years
Adverse Events		X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X

[a] see section **2.2** and section **3.3.1** for details

[b] see section **3.4.2** for details of testing during hospitalization

[c] only if cortisol<18 mg/dL or clinical suspicion of adrenal insufficiency

[d] bone marrow biopsy will include flow cytometry, BCMA expression, cytogenetics (pretreatment bone marrow aspirate only), amyloid staining (pretreatment bone marrow biopsy only), and molecular studies of each sample.

[e] Only if a plasmacytoma/lesion was detected before treatment

[f] if useful for response assessment

[g] RCR blood collection will only continue after the 1 year time-point if a previous RCR test has been positive, see section **3.6** for details

[h] required at baseline, only needed at screening if needed to evaluate disease..

[i] only D+30, no CTs or PETs indicated at D+14

[j] Patients who are known to be positive for any of these tests do not need to be retested; may be performed within 3 months of chemotherapy start date

[k] only at Screening

[l] Always do a bone marrow aspirate and biopsy on day 14; only do a bone marrow aspirate and biopsy on Day 30 if clinically indicated. Additional bone marrow biopsies and aspirates might be needed if clinically indicated due to low blood counts or for myeloma response assessment.

[m] Done Daily during hositalization

[n] In addition to baseline research blood, 1 SST tube must be drawn on the morning of CAR T-cell infusion prior to CAR T-cell infusion

[o] +/- 15 minutes

[p] Draw 1 SST tube on the first Sunday after CAR T-cell infusion. This SST tube should be stored refrigerated on the nursing unit until Monday morning when it should be processed first thing Monday morning at the latest.

[q]A bone marrow aspirate and biopsy with flow cytometry will also be performed to confirm suspected complete remission. Note that 2 separate bone holes will be required for the pretreatment and 2-week follow-up bone marrow aspirates. All other bone marrow aspirates will only require 1 bone hole.

[r] In addition to the day 0 and 1st Sunday research blood mentioned above, research blood is drawn every Mon-Wed-Fri while the patient is inpatient.

[s]Weight at admission is adequate even if not D0. Height is only required to be performed sometime before starting chemotherapy.

[t] only D+14

[u] only if needed to stage MM

[v] Bone marrow aspirate and biopsy with flow cytometry will be perfomed at the 6-month, 18-month, 30-month, and 42- month time-points

3.6 GENE-THERAPY-SPECIFIC FOLLOW-UP

- Long-term follow up of patients receiving gene transfer is required by the FDA and must continue even after the patient comes off the treatment study. Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires for 10 additional years for a total of 15 years after cell infusion. Blood will need to be collected at least annually for long-term follow-up of gene therapy**.** After 5 years, patients who are still on this study will be transferred to the long-term gene therapy protocol NCI protocol 15-C-0141 to complete long-term gene-therapy follow-up.
- Persistence of CAR transduced cells will be assessed by quantitative PCR and/or flow cytometry at 1, 2, 3, 4, 6 and 12 months after cell infusion, or until CAR-expressing cells are no longer detectable. If any patient shows a high level of persistence of CAR gene transduced cells or an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells.
- Patients' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR prior to cell infusion and at 3, 6, and 12 months post cell administration. If all of these samples are negative for RCR, blood collection for RCR will be discontinued after the 12 month time-point..
- S+L- culture-based replication-competent retrovirus testing will be conducted on the infusion CAR T cells of all patients

3.7 COST AND COMPENSATION

3.7.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.7.2 Compensation

Participants will not be compensated on this study.

3.7.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days after the last dose of study therapy.

3.8.1 Criteria for removal from protocol therapy

Note that the treatment consists of a conditioning chemotherapy regimen followed by a T-cell infusion.

Patients will be taken off treatment for the following:

- Any DLT makes patients ineligible for repeat treatments.
- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen. If the reason that the patient is not eligible can be rapidly resolved within 2 weeks, the patient can proceed on treatment for up to two weeks, otherwise the patient must come off treatment and then off study as appropriate. An exception to this is that a platelet count of 50,000 or more is considered adequate to start chemotherapy.
- The patient started chemotherapy but cannot complete the entire treatment (ending with completed cell infusion) for any reason specified in the protocol or PI discretion. If the reason that the patient is no longer eligible can be rapidly resolved, the patient can proceed on treatment, otherwise the patient must come off treatment and then off study as appropriate.
- The patient receives any other treatment for multiple myeloma except bisphosphonates and repeat treatment on this protocol. Bisphosphonates are allowed when administered to prevent osteopenia more than 2 months after the anti-BCMA-CAR T-cell infusion.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

3.8.2 Off-Study Criteria

Patients will be taken off study for the following: (all patients coming off study will enroll on the long-term gene therapy follow-up protocol 15-c-0141)

- The patient completes the study upon reaching 5 years after CAR T-cell infusion.
- The patient completes off treatment follow-up
- The patient voluntarily withdraws
- There is significant patient noncompliance
- PI discretion
- Death

- PI decision to end this study
- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen.
- Development of progressive or relapsed multiple myeloma after anti-BCMA CAR T cell infusion in patients not desiring or not eligible for re-treatment on this protocol.
- The patient receives any anti-myeloma therapy except bisphosphonates. Bisphosphonates are allowed 2 months or more after the CAR T-cell infusion.

3.9 STOPPING CRITERIA

- If no responses of PR or CR occur after 2 patients are treated on the highest dose level, the protocol will be stopped.
- Instructions for how to proceed when toxicity occurs will be as instructed by the dose escalation section of the protocol.
- A death on study not attributible to progressive malignancy within 30 days of a cell infusion for the initial 5 subjects will be a cause for a pause to accrual pending discussion with the FDA and IRB.
- If 3 or more of the first 9 treated patients experience grade 4 toxicity possibly or probably attributable to CAR T cells within 30 days of cell infusion, this will be a cause for a pause to accrual to reassess the safety of the product pending amendment of the protocol approved/reviewed by the FDA and IRB.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 ANTIBIOTIC PROPHYLAXIS

- Patients with a CD4 T-cell count less than 200 will be maintained on pneumocystis prophylaxis with atovaquone or inhaled pentamidine. Patients with a CD4 T-cell count less than 200 will also be maintained on acyclovir or valacyclovir.

  Patients with serum IgG level less than 600 mg/dL will receive intravenous immunoglobulin replacement as needed to maintain an IgG level above 600 mg/dL after at least 1 month post-CAR T-cell infusion. An example of an intravenous immunoglobulin infusion to be used for this purpose would be Gammunex 500 mg/kg given as a single dose. Intravenous immunoglobulin infusions should be preceded by premedication with diphenhydramine and acetaminophen, and rate of infusion should be started at low rates and escalated in a step-wise manner
- Neutropenic patients will start on broad spectrum antibiotics with a first fever of 38.30C or greater or two fevers of 38.0 separated by at least 1 hour and concomitant ANC < 500/mL.
- Aminoglycosides will be avoided unless clear evidence of sepsis.

4.2 BLOOD PRODUCT SUPPORT

- Leukocyte filters will be utilized for all blood and platelet transfusions with the exception of the CAR-transduced T cell infusions to decrease sensitization to transfused WBC and decrease the risk of CMV infection.
- Patients who are seronegative for CMV should receive CMV-negative blood products whenever possible.
- Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hgb >8.0 gm/dL, and platelets >10,000/mm3. All blood products with the exception of the CAR-transduced T cells will be irradiated. Leukocyte filters will be utilized for all PRBC and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

4.3 ANTI-EMETICS

Anti-emetics will follow NIH Clinical Center Guidelines (except that corticosteroids will be avoided).

4.4 GRANULOCYTE COLONY-STIMULATING FACTOR

A CBC will be obtained daily while the patient is inpatient. If the absolute neutrophil count becomes less than 500/microliter, Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only in patients with absolute neutrophil counts less than 500/microliter. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.

4.5 AVOIDANCE OF CORTICOSTEROIDS

Patients should not take corticosteroids including prednisone, dexamethasone or any other corticosteroid at a dose equivalent to 5 mg/day or more of prednisone for any purpose without approval of the Principal Investigator.

4.6 TOXICITY MANAGEMENT GUIDLINES

Guidelines for management of common CAR toxicities including cytokine release syndrome (CRS) and neurologic toxicity are in section **15.3**.

5 BIOSPECIMEN COLLECTION

Biospecimen collection on this protocol will consist of blood draws and acquisition of bone marrow aspirates and possible biopsies of plasmacytomas for research purposes. Blood and bone marrow collection is described above in Sections **2.2, 3.4**, and **3.5**.

5.1 CORRELATIVE STUDIES FOR RESEARCH

Note regarding research Biospecimen collection: If any of the below time points fall on a federal holiday then, we may collect research blood on the next day.

Note: Platforms and procedures for analysis may be adjusted based upon current technology and/or collaborations in place at the time of actual analyses.

5.1.1 Biospecimen collection before the start of the conditioning chemotherapy:

- One heparinized syringe containing 2-2.5 mL of bone marrow aspirate to be sent to the Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755. It will be used in functional assays to see if anti-BCMA-CAR T cells can recognize the patient's multiple myeloma cells.
- Blood will be collected for research purposes. A total of 12 CPT tubes (8 mL each of blood will be collected prior to initiation of the conditioning chemotherapy regimen. This is a total of 96 mL of blood. Some of this blood will be used for immunology assays and some will be used for RCR assays. This blood can be collected on different days as long as a total of 12 CPT tubes are collected prior to the start of the chemotherapy and within 14 days of the start of the chemotherapy. Send to the Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755
- 16 mL of blood will be drawn to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 14 days prior to the start of the chemotherapy. Send to the BPC lab.
- An apheresis is required to obtain cells used to prepare the anti-BCMA CAR T cells that are administered on this protocol. After sufficient cells are processed for all possible clinical needs, the left over apheresis cells can be cryopreserved for research use. Note that these cells would simply be discarded if not used for research.
- Specimens will be cryopreserved and assays will be performed retrospectively.

5.1.2 Biospecimen collection Day 0 and after anti-BCMA-CAR T-cell infusion during the required hospitalization

Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the CAR T-cell infusion and lasting up until 14 days after infusion of anti-BCMA-CAR-transduced T cells, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Also, 1 SST tube (8 mL) will be drawn on the morning of CAR cell infusion prior to infusion of CAR T cells. 1 SST tube (8 mL) will be drawn on the first Sunday after CAR T-cell infusion. The 1 SST tube will be sent to BPC The 6 CPT tubes sent to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.

- Additional research blood may be collected at any time during the clinical course at the discretion of the PI (within the volume restriction limits) allowing for the research studies already outlined in this protocol to be performed at the time of an unanticipated clinical event, if necessary to address the objectives of the study.

5.1.3 Biospecimen collection during outpatient follow-up

- Patients will return for outpatient follow-up clinic visits 2 weeks, 1 month, 2 months, 3 months, 4 months, 6 months, 9 months and 12 months after the anti-BCMA- CAR T-cell infusion. After the 12-month follow-up appointment patients will return for follow-up every 6 months. The specimens listed below will be performed at each outpatient clinic visit during the first year of follow up.
    - 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Send CPT tubes to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.

- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. The SST tubes will be sent to BPC.

NOTE: After 1 year research blood collected will be reduced to 4 CPT tubes at each visit.

- At the 2 week, 2 month and 6 month, 18 month, 30 month and 42 month follow-ups, bone marrow aspirate and biopsy will be performed and the aspirate will be cryopreserved.

5.1.4 Immunological Testing

- T-cell assays: Direct immunological monitoring will consist of quantifying CD3+ T cells that express the anti-BCMA CAR by quantitative PCR, and/or by flow cytometry. These assays will be performed to measure the persistence and estimate the proliferation of the infused CAR+ T cells. A quantitative PCR assay or a flow cytometry assay will be used to quantitate CAR+ T cells at all post-infusion time-points up to at least 3 to 6 months after infusion, and CAR+ T cell analysis will continue until the CAR+ T cell level drops to undetectable levels unless a stable low level of CAR+ T cells is present at more than a year after infusion. The absolute number of CAR+ PBMC will be estimated by multiplying the percentage of CAR+ PBMC by the absolute number of lymphocytes plus monoctyes per microliter of blood. Ex vivo immunological assays will be used to measure the BCMA-specific functional activity of the CAR+ T cells and will consist of assays such as intracellular cytokine staining and anti-CD107a degranulation assays. Immunological assays will be standardized by the inclusion of pre-infusion recipient PBMC and in some cases an aliquot of the engineered T cells cryopreserved at the time of infusion.
- Studies of patient T-cell and antibody immune responses against the CAR will be carried out.
- Serum cytokine levels will also be measured by enzyme-linked immune sorbent assays.
- Gene expression studies might be performed on patient multiple myeloma cells and on the infusion CAR T cells of each patient. Methods used will be either Nanostring and/or RNAseq (RNA sequencing).
- Patients' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR **prior to cell infusion and at 3 and 6 months, and at one year post cell administration**. Monitoring for RCR will be discontinued if all patient samples have been negative for RCR at the 12 month time-point .. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the Gibbon Ape Leukemia Virus (GALV) envelop gene and are performed under contract by the National Gene Vector Laboratory at Indiana University The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch (SB) research team.
- Due to nature of these studies, it is expected that expansion of specific T-cell clones will be observed as T-cell proliferate in response to the targeted antigen. Therefore, care will be taken to track T-cell persistence, but presence of an oligoclonal T cell population does not indicate an insertional mutagenesis event. If any patient shows a high level of persistence of CAR gene transduced cells or an increasing population of CAR gene

transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from CAR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

5.1.5 Additional biopsies and additional blood draws

Patients might be asked to undergo biopsies or additional blood draws as clinically indicated. Additional blood draws might be necessary to investigate T cell responses and serum cytokine levels in cases of clinical events such as rapid regressions of malignancy or toxicity. . These biopsies will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count. Biopsy tissue will be processed in the NCI Laboratory of Pathology. Remainder material from such biopsies studies will be performed to evaluate the antigen expression by the tumor and to evaluate the reactivity of lymphocyte from these biopsies. In addition the presence of transduced cells may be quantitated.

5.1.6 Future studies

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study the patient provides consent for this. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g. analysis of germ line genetic mutations) a protocol amendment will be required and informed consent will be obtained from all research subjects to whom these new studies and risks pertain.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.2.1 Sample Tracking and Processing

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. All samples will be sent to Biospecimen Processing Core (BPC) and/or SB-CPF for processing and/or and storage until they are distributed to the designated place of analysis as described in the protocol. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.2.1.1 Biospecimen Processing Core (BPC)

5.2.1.1.1 Samples Sent to the BPC Lab

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research.

- Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.

After delivery to the SB-CPF, peripheral blood mononuclear cell samples will be sent to the Head, Clinical Support Laboratory Clinical Services Program, Applied/Developmental Directorate Frederick National Laboratory for Cancer Research for processing and cryopreservation. They will be stored long-term at the NCI Frederick Repository.

5.2.1.1.2 BPC Contact Information

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main BPC number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.2.2 Sample Storage and Disposition

All samples sent to the Biospecimen Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to specified computers . These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. Data will be recorded for each sample as applicable (e.g. the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location). Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked inLabmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB-approved protocol) and that any unused samples

must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.2.2.1 Sample Storage, Tracking, and Disposition for Surgery Branch

Samples received by the Surgery Branch research lab will be tracked using password protected web-based NCI database Labmatrix. All specimens will be tracked for date of receipt in the Surgery Branch lab, date analyzed, date returned to the originating hospital and/or date destroyed. Specimens will be stored in a locked laboratory cabinet or refrigerators in a locked research lab. All specimens will be entered into Labmatrix with identification and storage location. Access to the stored specimens will be restricted. Access to Labmatrix will be granted upon PI approval only. It is the responsibility of the NCI PI to ensure that the specimens are being used and stored in a manner consistent with IRB approval. All samples are stored in monitored freezers/refrigerators in 3NW NCI-SB laboratories at specified temperatures with alarm systems in place.

5.2.3 Protocol Completion/Sample Destruction

Any specimens remaining at the completion of the protocol will be stored indefinitely in the conditions described above. The study will remain open so long as sample or data analysis continues. All samples and data from consenting participants will be stored in identifiable format until they are no longer of scientific value or if a participant withdraws consent for their continued use, at which time they will be destroyed.

If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed or returned to the participant if so requested. The participant's samples and data will be excluded from future distributions, but those which have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of section **7.2**.

5.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

Samples for gene expression analysis will use RNAseq and/or Nanostring™. These studies will be used to determine gene expression in multiple myeloma cells and infusion CAR T cells. The purpose of these studies is to assess gene expression at the RNA level not to study germline mutations.

5.3.1 Description of the scope of genetic/genomic analysis

RNAseq and/or Nanostring™ will be used to determine gene expression in multiple myeloma cells and infusion CAR T cells. The purpose of these studies is to assess gene expression at the RNA level not to study germline mutations. One purpose of these studies is to determine if different levels of gene expression in malignant cells are associated with response to CAR T-cell therapy. Another purpose of these studies is to determine if different levels of gene expression in infusion CAR T cells are associated with anti-malignancy responses caused by CAR T cells or persistence of CAR T cells.

5.3.2 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study has obtained a Certificate of Confidentiality, which helps to protect personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation. The informed consent includes the appropriate coverage and restrictions of the Certificate of Confidentiality.

5.3.3 Management of Results

The analysis that we perform in our laboratory are for research purposes only; they are not nearly as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing. Changes that we observe unrelated to our research may or may not be valid. Therefore, we do not plan to inform participants of the results of testing on the tissue and blood that is performed in our research lab. However, subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists) Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. The CLIA testing may be funded by the PI or the CCR. If the research findings are verified in the CLIA certified lab, the subject will be referred to the NCI Genetics Branch for genetic counseling on the implications of the results. Subjects that do not wish to return to the NCI will be referred to a local genetics health care provider (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data will be prospectively collected and entered into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The NCI PI, research nurse, and designated members of the research team will have access to these data via web access. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

The medical record will maintain complete records on each patient including any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records. The NCI study database will serve as the primary source from which all research analyses will be performed.

Data collection will include the patient history, specialty forms for pathology, radiology, toxicity monitoring, and relapse data and an off-study summary sheet, including a final assessment by the treating physician.

All AEs, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after the study treatment was last administered. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded, unless otherwise noted in Section 6.1.1.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Adverse event recording

- Grade 1 adverse events will not be recorded.
- All Grade 2 events will be recorded regardless of attribution up to at least 30 days after CAR T-cell infusion
- After 30 days after CAR T-cell infusion Grade 2 adverse events that will be recorded:
    a. Unexpected events that are possibly, probably, or definitely related to the research.
    b. Expected events that are probably or definitely related to the study interventions will be recorded only for the first year after the infusion.
    c. All Infections proven by culture, PCR, antigen detection or other laboratory methods will be recorded for the first year after infusion regardless of attribution.
    d. Any serious events that are deemed clinically significant by the PI
- All grade 3, 4, and 5 adverse events will be recorded regardless of attribution.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

I will share human data generated in this research for future research as follows:

_x__ Coded, linked data in an NIH-funded or approved public repository.

_x__ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_x__ Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

_x__ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov.

_x__ BTRIS (automatic for activities in the Clinical Center)

_x__ Approved outside collaborators under appropriate individual agreements.

_x__ Publication and/or public presentations.

When will the data be shared

_x__ Before publication.

_x__ At the time of publication or shortly thereafter.

6.3 GENOMIC DATA SHARING PLAN

Unlinked genomic data will be deposited in the database of genotypes and phenotypes (dbGaP) in compliance with the NIH Genomic Data Sharing Policy.

6.4 RESPONSE CRITERIA

Responses will be categorized by using the International Uniform Response Criteria for Multiple myeloma 2016 updated version.[111] Multiple myeloma staging will be conducted at the 2 week follow-up appointment and at each subsequent follow-up appointment. The appropriate staging studies will need to be determined for each patient because of the variability in multiple myeloma.

6.4.1 Important Considerations on response criteria

- Response criteria for all categories and subcategories of response except CR and sCR are applicable only to patients who have 'measurable' disease by at least one of the three measurements as defined below
- All responses must be confirmed to be stable in two evaluations made at any time.

6.4.1.1 Definition of measurable disease[16]

Any one or combination of these abnormalities defines measurable disease:

- Serum M-protein greater or equal to 1.0 g/dL (10 g/L).
- Urine M-protein is greater or equal to 200 mg/24 h.
- Serum FLC assay: Involving FLC level greater or equal to10 mg/dL (100 mg/L) provided serum FLC ratio is abnormal.
- Presence of a biopsy-proven plasmacytoma
- Bone marrow plasma cells >30% of total bone marrow cells

6.4.1.2 Laboratory tests for measurement of M-protein

- Serum M-protein level is quantitated using densitometry on SPEP except in cases where the SPEP is felt to be unreliable such as in patients with IgA monoclonal proteins migrating in the beta region. If SPEP is not available or felt to be unreliable (e.g., in some cases of IgA myeloma) for routine M-protein quantitation during therapy, then quantitative immunoglobulin levels on nephelometry or urbidometry can be accepted. However, this must be explicitly reported, and only nephelometry can be used for that patient to assess response and SPEP and nephelometric values cannot be used interchangeably.

- Urine M-protein measurement is estimated using 24-h UPEP only. (Random or 24 h urine tests measuring kappa and lambda light chain levels are not reliable and are not recommended)

6.4.1.3 Suggested follow-up to meet response criteria

- Patients with measurable disease by both SPEP and UPEP need to be followed by both SPEP and UPEP for response assessment and categorization;
- Except for assessment of sCR, CR, and VGPR, patients with "measurable disease" restricted to the SPEP will need to be followed routinely only by SPEP;
- Patients with "measurable disease" restricted to the UPEP will need to be followed routinely only by UPEP;
- Patients with "measurable disease" in either SPEP or UPEP or both will be assessed for response only based on these two tests and not by the FLC assay;
- FLC response criteria are only applicable to patients without measurable disease in the serum or urine, and to fulfill the requirements of the category of sCR;
- Bone marrow is required only for categorization of CR
- For good clinical practice patients should be periodically screened for light chain escape with UPEP or serum FLC assay.
- Plasmacytomas can be staged at baseline and followed for response with CT scans by measuring target lesions with the sums of the products of the diameters method. For purposes of defining CR, masses greater than 2.0 cm will be considered abnormal.

6.4.2 International Myeloma Working Group uniform response criteria:

6.4.2.1 Stringent Complete Remission (sCR)

- CR as defined below plus
  - Normal FLC ratio and
  - Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence or flow cytometry (only1 bone marrow evaluation is needed).

6.4.2.2 Complete Remission (CR)

- Negative immunofixation on the serum and urine **and**
- Disappearance of any soft tissue plasmacytomas **and**
- 5% or less plasma cells in bone marrow (only 1 bone marrow evaluation is needed)
- No evidence of progressive or new bone lesions if radiographic studies were performed

  (X-Rays not required in absence of clinical indication)
  Comments: To be considered a CR,
- **Both** serum and urine immunofixation must be carried out and be negative regardless of the size of baseline M-protein in the serum or urine;

- Patients with negative UPEP values pretreatment still require UPEP testing to confirm CR and exclude light chain or Bence–Jones escape

6.4.2.3 Very Good Partial Remission (VGPR)

- Serum and urine M-protein detectable by immunofixation but not on electrophoresis **or**
- 90% or greater reduction in serum M-protein **plus** urine M-protein level <100mg per 24 h
- No evidence of progressive or new bone lesions if radiographic studies were performed (X-Rays not required in absence of clinical indication)
- 90% decrease in the sum of the products of the the diameters of soft tissue plasmacytomas is required.

6.4.2.4 Partial Remission (PR)

- 50% or greater reduction of serum M-protein **and**
- 90% or greater reduction in 24-h urinary M-protein (or to less than 200mg per 24 h) **and**
- 50% or greater reduction in the size of soft tissue plasmacytomas, if present at baseline
- No evidence of progressive or new bone lesions if radiographic studies were performed (X-Rays not required in absence of clinical indication)

Only if the serum and urine M-protein are not measurable (as per definition in section **6.4.1.1**),

- 50% or greater decrease in the difference between involved and uninvolved FLC levels is required (in lieu of the serum and urine M-protein criteria).

6.4.2.5 Stable Disease (SD)

- Not meeting criteria for CR, VGPR, PR or progressive disease

(Not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates)

- All response categories require two consecutive assessments made at **any time** before the institution of any new therapy;
- Confirmation with repeat bone marrow biopsy not needed.
- Presence/absence of clonal cells is based upon the κ/λ ratio. An abnormal κ/λ ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is κ/λ ration of greater than 4:1 or less than 1:2.

6.4.2.6 Progressive Disease (PD)[a]

Requires one or more of the following:

- Increases of greater or equal to 25% from the lowest post-treatment (nadir) value in
  - Serum M-component (minimum absolute increase of 0.5g/dL) or
  - Urine M-component (minimum absolute increase of 200mg/24h) or

- Percentage of bone marrow plasma cells (minimum absolute percentage of 10%)
- Only in patients without measurable serum and urine M-protein levels: The difference between involved and uninvolved FLC levels. The absolute increase must be >10 mg/dL. The FLC ratio must be abnormal.

- Definite development of new bone lesions or clear increase in size of on or more bone lesion(s) (must be 50% or greater increase of a lesion at least 1 cm in longest length at nadir) on CT scan or new plasmacytoma 2.0 cm or larger on CT scan.
- 50% or more increase in the sum of the products of the diameters of multiple soft tissue plasmacytomas or 50% or more increase in the size of a single soft tissue plasmacytoma.
- Development of hypercalcemia solely attributable to the disease (corrected serum calcium >11.5 mg/dL)

[a]All relapse categories require 2 consecutive assessments made at any time before classification as relapse or progression or institution of a new therapy.

6.4.3 Minimal residual disease (MRD) criteria (requires complete response as defined above)

- Sustained MRD-negative MRD negativity in the marrow (next generation flow (NGF) or next generation sequencing (NGS) or both) and by imaging as confirmed at least with assessments at least 1 year apart.
- Flow MRD-negative-Absence of aberrant clonal plasma cells in the bone marrow by NGF using Euro Flow standard operating procedures or equivalent; must have sensitivity of one in 10^5 nucleated cells.
- Sequencing MRD-negative-absence of clonal plasma cells by NGS on bone marrow aspirate with a method with sensitivity of 1 in 10^5 nucleated cells.
- Imaging plus MRD-negative-MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less than mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue.

6.5 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/hrpp-policy-guidelines/

7.2 OHSRP Office of Compliance and Training / IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/hrpp-policy-guidelines/ Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/hrpp-policy-guidelines/.

7.3 NCI Clinical Director Reporting

Problems expeditiously reported to the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee. A separate submission is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 Institutional Biosafety Committee (IBC) Reporting Criteria

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of anti-BCMA CAR T cells as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the anti-BCMA CAR-expressing T cells, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Additional Information to IBC

Refer to the current IBC reporting requirements to provide additional information to the IBC, including any related to annual/progress reports, as applicable.

7.5 Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator.

Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines. The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator

will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/ SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section 6.1. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets a protocol-defined serious criterion or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section 8.2.

SAE reports will be submitted via an electronic SAE reporting system (e.g., HiLIT). In the event of system downtime or issues , SAE reports will be submitted using the CCR SAE Report form to the sponsor at: OSROSafety@mail.nih.gov. CCR SAE report form and instructions can be found at: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI [*add if multicenter:* at NIH or request from your study coordinator for non-NIH

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions.

8.4.1 Maternal exposure

If a patient becomes pregnant during the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.4.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of receiving protocol treatment.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies occurring from the date of the first dose until (*120 days)* after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.5 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected . CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all

investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

The primary endpoint of this trial is to determine the safety of administering anti-BCMA-CAR-expressing T cells to patients with multiple myeloma. The primary approach for assessing this endpoint will be through a dose escalation.

Exploratory objectives of this trial are to measure any anti-malignancy effect that might occur, to assess the feasibility of administering anti-BCMA-CAR-expressing T cells, and to measure persistence, function, and gene expression of anti-BCMA-CAR-expressing T cells.

10.2 SAMPLE SIZE DETERMINATION

The sample size of this clinical trial will be determined by the requirements of the dose-escalation scheme. A maximum of 30 treated patients will be needed to complete the dose escalation scheme, and an additional 12-patient expansion group will be treated at the MTD. The 12-patient expansion group to better characterize the MTD is needed because of the substantial variability between patients receiving infusions of CAR T cells. The total number of treated patients will be a maximum of 42 including patients who may be re-enrolled. We estimate that 120 patients will need to be screened at the NIH to recruit up to 42 treated patients.

The trial uses a dose-escalation design, with 5 dose levels and a -1 dose level if needed. The number of anti-BCMA-CAR-expressing T cells transferred for each dose level is as follows:

Dose level -1	$0.4x10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 1	$0.75x10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 2	$1.5x10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 3	$3.0x10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 4	$6.0x10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 5	$12.0x10^6$ CAR+ T cells per kg of recipient bodyweight

Patients will be enrolled sequentially; therefore, enrollment will not proceed to a higher dose level until all patients have been treated on the prior dose level. If sufficient cells cannot be grown to meet the criteria for the assigned dose level, the patient will receive the dose of cells called for by one dose level lower than the assigned dose level. If sufficient cells cannot be grown to meet the dose requirement called for one dose level lower than the assigned dose level, the treatment will be aborted. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at this level as described in the dose-escalation scheme in section **3.1.4**. Accrual will be halted at the higher level until accrual at the lower level is complete as described above.

Should none of the first 3 patients treated on a dose level experience a DLT, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the third patient. Should 1 of 3 patients experience a DLT at a particular dose level, three more patients would be treated at that dose level. If 1/6 patients have a DLT at a particular dose level, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the 6^{th} patient.If a level with 2 or more DLTs in 3-6 patients has been identified, 3 additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the MTD. The MTD is the dose at which a maximum of 1 of 6 patients has a DLT. After a MTD is defined, additional patients can be treated on this trial: up to 12 total additional recipients can be treated after a MTD is established in order to better define safety and toxicity at that dose level. If treatment of at least 3 patients on each dose level is completed without establishing a MTD, a maximum feasible dose can be declared, and up to 12 additional patients can be treated at the maximum feasible dose level. If cell growth limitations preclude administration of the maximum tolerated dose to a patient in the expansion group, the patient will receive as many cells as possible up to the MTD.

DLTs that occur in patients receiving a repeat treatment will not affect the dose escalation of patients receiving an initial treatment. However, excessive DLTs among re-treatments will result in a discontinuation of this practice. Specifically, if 2 of the first 3 patients re-treated, 3 or more of the first 6 re-treated patients, 4 or more out of the first 9 re-treated patients, or greater than 1/3 of the total patients receiving a repeat treatment experiences a DLT during repeat treatment, then repeat treatments will be discontinued altogether.

10.3 POPULATIONS FOR ANALYSES

All treated patients will be included in the safety analysis dataset.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

This is a standard 3+3 dose escalation study. Toxicity data will be obtained and used to determine the MTD of the treatment.

10.4.2 Analysis of the Primary Endpoints

The toxicity obtained on each patient will be determined and used to report the number of patients at each dose level who experience a DLT, following standard phase I procedures.

10.4.3 Analysis of the Secondary Endpoint(s)

There are no secondary endpoints.

10.4.4 Safety Analyses

The study will determine the toxicity experienced on each patient enrolled on the trial and report this by dose level.

10.4.5 Baseline Descriptive Statistics

None will be provided in a formal manner; brief descriptions may be incorporated in a final report.

10.4.6 Planned Interim Analyses

Toxicity will be evaluated at each dose level as the patients accrue to the trial. Stopping criteria are described in section **3.9.**

10.4.7 Sub-Group Analyses

None

10.4.8 Tabulation of individual Participant Data

None

10.4.9 Exploratory Analyses

The degree of persistence of anti-BCMA-CAR-transduced T cells will be evaluated by a quantitative measure (flow cytometry and/or quantitative PCR) in all patients. Serum cytokine levels and T-cell gene expression levels are other important exploratory assessments. Anti-malignancy effects will be measured by clinical response and categorized according to the International Uniform Response Criteria for Multiple Myeloma (Section **6.4**). The clinical multiple myeloma responses will be interpreted cautiously in the context of a pilot study which may be used to guide parameters for study in future protocols if warranted.

All other evaluations of exploratory objectives will be performed using exploratory techniques. No formal adjustment for multiple comparisons will be used since the evaluations are being done to generate hypotheses. Exploratory assessments will include analysis for associations between

clinical outcomes such as severity of toxicity and serum cytokine levels or severity of toxicity and blood CAR T-cell levels. Parametric and nonparametric tests will be used as appropriate.

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

- The patients to be entered in this protocol have multiple myeloma which is an almost always incurable disease; moreover, patients will have progressive or relapsed myeloma despite at least 3 prior therapies. These patients have limited life expectancies. Subjects from both sexes and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore sex and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to sex or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.
- MM remains an incurable disease despite recent advances in therapy.
- Over the last 40 years and throughout the most recent era of improved therapy with novel agents, the depth of the disease response to therapy (including T- cells targeting B-cell maturation antigen) has remained the single most predictive factor for event free and overall survival in MM
- Therefore, improving the rate and depth of responses remains a high priority for clinical research in MM.
- This justifies the enrollment of all subjects with MM eligible for T- cells targeting B-cell maturation antigen in this phase I/II study in an attempt to improve the clinical outcome while feasibility and safety are being evaluated.
- Because patients on previous trials of CAR T cells have experienced hypotension, tachycardia, prolonged fevers, neurological toxicities, and depressed myocardial function. In many cases these toxicities were severe enough to require intensive care unit admission. We will limit enrollment to patients 70 years of age or less because based on our admittedly limited experience with prior CAR-T cell clinical trials, younger patients tolerate and recover from these toxicities better than elderly patients.

11.2 PARTICIPATION OF CHILDREN

Children will not be enrolled on this study. Multiple myeloma is extremely uncommon in children; moreover, since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit.

11.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from

research participation (section **11.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed. Please see section **11.5.1** for consent procedure.

11.4 Evaluation of Benefits and Risks/Discomforts

The experimental treatment has a chance to provide clinical benefit though this is unknown. A goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using genetically-modified T-cells. This specific protocol is being performed to evaluate a genetically modified T-cell therapy for multiple myeloma, which is an almost always incurable disease.

Only patients with multiple myeloma who have progressive or relapsed myeloma despite at least 3 prior lines of therapy will be enrolled.

The risks of the study fall into 3 general categories (see section **12** for details). First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release type toxicities such as high fevers, tachycardia, hypotension and neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. These cytokine-release-type toxicities have been detected in other clinical trials of CAR T cells during the first 2 weeks after anti-BCMA CAR T cells were infused.[4,107] The third main category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected expression of BCMA on normal cells or because of unexpected cross-reactivity of the anti-BCMA CAR with proteins other than BCMA in vivo. Another potential toxicity of anti-BCMA CAR T cells is hypogammaglobulinemia due to depletion of plasma cells and mature B cells. Hypogammaglobulinemia has been a complication of many patients on clinical trials of anti-BCMA CAR-expressing T cells.[4,33] Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[4]

The gammaretroviral vector used in this trial inserts into the T-cell DNA of patients, so in theory, insertional mutagenesis could occur, but insertional mutagenesis has not occurred in any of the hundreds of patients treated with mature T cells that were genetically modified by gammaretroviral vectors.[35-37]

The success of this clinical trial cannot be predicted at this time. Because all patients in this protocol have advanced multiple myeloma and limited life expectancies the potential benefit is thought to outweigh the potential risks. It is also anticipated that this study will provide scientific information relevant to tumor immunotherapy.

11.4.1 Risk to family or relatives

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems. Patients will be

notified of any medically significant and actionable incidental findings. Study results will not be shared with patients.

11.4.2 Risks of exposure to Ionizing Radiation

The procedures for performing the CT scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive additional radiation exposure from up to eight (8) CT head+ Neck +CAP and five (5)18FDG-PET/CT scans in the first year of the study.

The total additional radiation dose for research purposes will be approximately 16.9 rem in the first year of the study. This amount is more than would be expected from everyday background radiation. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 1.7 out of 100 (1.7%) and of getting a fatal cancer is 0.8 out of 100 (0.8%).

11.4.3 Risks of Scans and Contrast

If contrast dye is used, there is a small chance of developing an allergic reaction from the contrast material including gadolinium, which may cause symptoms ranging from mild itching or a rash to severe difficulty breathing, shock or rarely, death. The contrast material may also cause kidney problems. Gadolinium for research MRI scans will not be given to patients who have impaired kidney function or who received gadolinium within the previous month.Common reactions include pain in the vein where the contrast was given, a metallic or bitter taste in the mouth , headache, nausea and a warm or flushing feeling that lasts from 1-3 minutes.

An IV line may need to be inserted for administration of the contrast agent or anesthetic, which may cause pain at the site where the IV is placed and there is a small risk of bruising or infection

11.4.4 Risks of blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

11.4.5 Risks of Bone marrow aspiration and biopsy

Side effect of bone marrow aspiration and biopsy may feel a pressure sensation when the needle is being inserted and a pulling sensation and brief pain as the marrow is withdrawn. Potential complications of this procedure are local bleeding, pain at the site, and infection. Both of these are very rare. Bleeding can be stopped by applying local pressure and an infection can be treated with antibiotics.

11.4.6 Risks of Intravenous Catheter

Side effect of placing some catheters include pain, bleeding, infection and rarely, collapsed lung. The long- term risks of the catheter rarely include infection and clotting of veins.

11.4.7 Risks of Apheresis

The risks of apheresis are similar to whole blood donation and include pain and bruising at the needle insertion site in the arms, lightheadedness, dizziness, nausea, and rarely fainting due to a rare reflex reaction to needle placement and to the temporary decrease in blood volume during apheresis. It may also feel tingling around your mouth or in your fingers caused by a blood thinner

given during the procedure. The tingling may reduce by giving calcium containing chewable antacid. All the symptoms usually go away within a few minutes of stopping the procedure.

11.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided to the participant or consent designee(s) (e.g., legally authorized representative [LAR] if participant is an adult unable to consent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with local policy, including HRPP policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

11.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **11.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **11.5**.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the National Cancer Institute Center for Cancer Research (NCI CCR). This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

Note: The commercial drugs used in this study will not alter labelling of the FDA approved drugs and nor does the investigation involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

13.1 RETROVIRAL VECTOR CONTAINING THE ANTI-BCMA CAR GENE

13.1.1 Cells manufacturing

The retroviral vector supernatant (MSGV-FHVH33-CD8BBZ) encoding a CAR directed against BCMA was prepared and preserved following cGMP conditions in the University of Cincinnati Medical Center Vector Production Facility. The retroviral vector utilizes the MSGV retroviral vector backbone and consists of 7007 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, the anti-BCMA CAR protein containing a signal peptide from human CD8-alpha signal sequence, the fully-human heavy chain variable region 316833 (FHVH33), CD8 (hinge and transmembrane), CD28 (cytoplasmic region), and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR.

The supernatant will be stored at –80^{o}C or shipped on dry ice and stored in the Dept. of Transfusion Medicine, NIH or at FisherBiosciences, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transductions of T cells. There will be no re-use of the same unit of supernatant for different patients. Retroviral titer has been shown to be stable after immediate thawing and

immediate use. Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm

13.1.2 Toxicities

Please refer to section 1.2.11

13.1.3 Administration procedures:

Please see section **Error! Reference source not found.**.

13.2 COMMERCIAL AGENTS

Please refer to the US approved package insert for the full prescribing information here: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails

13.2.1 Cyclophosphamide

13.2.1.1 Source

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

13.2.1.2 Administration procedures

The cyclophosphamide used in this regimen will be given as Intravenous infusion over 30 minutes.

13.2.2 Fludarabine

13.2.2.1 Source

Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludarabine is stored at room temperature.

13.2.2.2 Administration procedures

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over 15 to 30 minutes.

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15 APPENDICES

15.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale*	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

15.2 APPENDIX B: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

All of the following elements will be recorded in the study database:

A. Patient Enrollment

- Date of birth, age, sex, race, ethnicity
- Height
- Weight
- ECOG
- Date of original diagnosis
- Stage at diagnosis
- Plasmacytoma present: Yes or No
- Tumor Histology and date of confirmation
- BCMA expression by tumor type of tissue studied and date of confirmation
- Date of Informed Consent signature, consent version and date of registration
- Baseline History/Physical
- Baseline Symptoms
- Prior therapy
- Prior radiation
- Prior Bone Marrow Biopsy that includes the percent of plasma cells and BCMA results
- Findings of consultations done at screening

B. Study Drug administration and response for each course of therapy given

- Dates anti-BCMA-CAR-transduced T cells given
- Dose level, actual dose in CAR+ T cells/kg, schedule and route given
- Height, weight, and body surface area at start of each course
- Response assessment for each restaging performed
- Concomitant medications will not be collected in the study database

C. Laboratory and Diagnostic Test Data

1. All Clinical laboratory and diagnostic test results done at screening and until day 30 post infusion with the following exceptions:
   Diagnostic tests which are not specified in the protocol, and if the results are not needed to document the start or end of an adverse event that requires reporting.
   Serologies such as CMV, HSV, EBV, toxoplasmosis, adenovirus,, TTV data that were not need for eligibility will not be collected.
2. All staging studies including serum protein electrophoresis, urine protein electrophoresis, serum free light chains, bone marrow biopsy reports, flow cytometry reports, serum immunoglobulin reports including beta-2 microglobulin, complete blood count and differential reports, serum creatinine reports, TBNK results, serum protein, urine albumin, serum calcium (including ionized), ESR and

CRP results. MRI, X-ray (including skeletal survey), and CT scan results will only be reported if they were used for staging.

D. Adverse Events

Please see section **6.1.1**Adverse Event Recording

E. Tumor response and measurements

- Restaging studies performed at protocol specified time points and as clinically indicated.
- Any physical exam findings will be collected as Adverse Events and labs results.
- Years 5-15 follow-up is only for survival.

F. Off study

- Date and reason for off study
- Date and cause of death
- Autopsy findings if available

15.3 APPENDIX C: GUIDELINES FOR MANAGEMENT OF COMMON TOXICITIES THAT OCCUR AFTER CAR T-CELL INFUSIONS

Infusions of CAR T cells are often complicated by significant acute toxicities in the first 2 to 3 weeks after the infusion. In many cases the toxicities correlate with serum inflammatory cytokine levels.

The toxicities most often experienced by patients receiving infusions of CAR T cells include, but are not limited to, tumor lysis syndrome, fever, fatigue, hypotension, tachycardia, acute renal failure, and neurological toxicities such as aphasia, ataxia, headache, somnolence, and coma. Fever is usually the first toxicity to occur.

Note these are guidelines that might require modification based on clinical circumstances of each patient, and failure to exactly follow these guidelines is not a protocol deviation or violation.

Administration of corticosteroids should be avoided if possible to avoid killing or impairing the function of the CAR T cells.

General supportive care guidelines

1. All patients with significant malignancy burdens and without a contradiction such as allergy should be started on allopurinol at the time of the start of the chemotherapy conditioning regimen or 1 day before the CAR T cell infusion. The suggested allopurinol dose is 200 to 300 mg/day with a possible loading dose of 300 to 400 mg.
2. Vital signs should be checked a minimum of every 4 hours during hospitalization. Increasing the time interval between vital sign checks for patient convenience or other reasons should be avoided.
3. Strict ins and outs should be recorded on all patients.
4. As a minimum, keep hemoglobin greater than 8.0 g/dL and platelets greater than 20K/microliter.
5. Administer fresh frozen plasma (FFP) for a PTT 1.5-fold or more above the upper limit of normal.
6. For patients with an increased PTT, check the fibrinogen level and keep the fibrinogen level above 100 mg/dL with cryoprecipitate.
7. Fevers should be treated with acetaminophen and comfort measures. NSAIDs and corticosteroids should be avoided.
8. Patients with a heart rate persistently higher than 115/minute and fever should have vital signs checked every 2 hours.
9. Patients who are neutropenic and febrile should be receiving broad-spectrum antibiotics.
10. Avoid meperidine due to seizure risk.
11. Minimize benzodiazepine use to avoid aggravating delirium.

12. Patients on this protocol will be placed on strict fall precautions including instructions to get out of bed only with assistance under the following conditions:
    1. Any history of syncope or near-syncope within 1 month before CAR T-cell infusion or any time after CAR T-cell infusion.
    2. Any blood pressure reading of less than 100 mm Hg systolic blood pressure after anti-CD19 CAR T-cell infusion if 100 mm Hg is lower than the patients baseline systolic blood pressure.
    3. Heart rate greater than 100 beats per minute.
13. Any patient with syncope, near-syncope, or light-headedness will have orthostatic blood pressure and heart rate checked and receive intravenous fluids as appropriate. These patients will also receive an ECG.
14. A CBC will be obtained twice daily while the patient is inpatient. If the absolute neutrophil count becomes less than 500/microliter, Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only in patients with absolute neutrophil counts less than 500/microliter. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.
15. Hypotension is a common toxicity requiring intensive care unit (ICU) admission. In general patients should be kept well-hydrated. Maintenance I.V. fluids (normal saline (NS) should be started on most patients with high fevers especially if oral intake is poor or the patient has tachycardia. I.V. fluids are not necessary for patients with good oral intake and mild fevers. For patients who are not having hypotension or tumor lysis syndrome, a generally even fluid balance should be strived for after allowing for insensible fluid losses in patients with high fevers. The baseline systolic blood pressure is defined for this protocol as the average of all systolic blood pressure readings obtained during the 24 hours prior to the CAR T-cell infusion. The first treatment for hypotension is administration of IV NS boluses.
    - **Patients with a systolic blood pressure that is less than 80% of their baseline blood pressure <u>and</u> less than 100 mm Hg should receive a 1 L NS bolus.**
    - **Patients with a systolic blood pressure less than 90 mm Hg should receive a 1 L NS bolus if this is lower than their baseline blood pressure.**

    These I.V. fluid management suggestions may need to be modified based on the clinical characteristics of individual patients such as pulmonary status, cardiac function, edema and other factors.
16. Patients receiving more than 1 fluid bolus for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram as soon as possible.

ICU transfer

Patients should be transferred to the ICU after consultation with the ICU physicians under these circumstances. Patients not meeting these criteria could also require ICU admission at the discretion of the clinical team caring for the patient.

- Systolic blood pressure less than 70% the patient's baseline blood pressure and less than 100 mm Hg after administration of a 1L NS bolus.
- Anytime the systolic blood pressure is less than 90 mm Hg after a 1L NS bolus.
- Continuous tachycardia with a heart rate higher than 125 beats per minute on at least 2 occasions separated by 2 hours.
- Oxygen requirement of more than a 4L standard nasal cannula

1. All patients transferred to the ICU for hypotension or tachycardia should have a stat EKG and a cardiac echocardiogram within 6 hours of the time of transfer.
2. Patients with hypotension not responding to IV fluid resuscitation should be started on norepinephrine at doses called for by standard ICU guidelines.
3. Patients should have a cardiac echocardiogram and an EKG within 6 hours of starting norepinephrine.
4. Patients in the ICU should get twice-daily labs (CBC with differential, acute care panel, mineral panel, hepatic panel, uric acid, LDH. Patients in the ICU should also get a daily troponin level).
5. Patients receiving vasopressors should have a cardiac echocardiogram at least every other day.

Immunosuppressive drug administration

In general, immunosuppressive drugs are administered in a stepwise escalation based on toxicity severity. The first immunosuppressive drug administered is usually tocilizumab. If toxicity does not improve after tocilizumab, treatment progresses to intermediate-dose or high-dose methylprednisolone. For certain severe toxicities listed below, high-dose methylprednisolone must be given immediately.

Tocilizumab administration

Tocilizumab should be administered under the following circumstances if the listed disorders are thought to be due to cytokine release from CAR T cells. Tocilizumab is administered at a dose of **8 mg/kg** infused IV over 1 hour (dose should not exceed 800 mg).

- Left ventricular ejection fraction less than 45% by echocardiogram
- Creatinine greater than 2-fold higher than the most recent level prior to CAR T-cell infusion
- Norepinephrine requirement at a dose greater than 3 μg/minute for 36 hours since the first administration of norepinephrine.
- Requirement of 5 mcg/minute or more of norepinephrine to maintain systolic blood pressure greater than 90 mm Hg.
- Oxygen requirement of 40% or greater fraction of inspired oxygen (FIO_2) to maintain oxygen saturation of >92%.

- Subjective significant dyspnea and respiratory rate greater than 25 for 2 hours or more.
- PTT or INR>2x upper limit of normal
- Bleeding possibly related to cytokine-release syndrome
- Creatine kinase greater than 5x upper limit of normal

Intermediate-dose methylprednisolone for toxicities not responsive to tocilizumab

1. Give methylprednisolone 50 mg every 6 hours for any of the toxicities under #19 above that don't improve after tocilizumab administration.

High-dose methylprednisolone should be given <u>immediately</u> under these circumstances:

1. Give methylprednisolone 200 mg every 6 hours for systolic blood pressure that is less than 90 mm Hg while the patient is on 15 mcg/minute or higher doses of norepinephrine. If the patient has not had tocilizumab, give 8 mg/kg of tocilizumab along with the methylprednisolone.
2. Give methylprednisolone 200 mg every 6 hours for hypotension requiring 15 mcg/minute or more of norepinephrine continuously for 8 hours or more. If the patient has not already had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
3. Give methylprednisolone 200 mg every 6 hours for any left ventricular ejection fraction 30% or less. If the patient has not already had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
4. Give methylprednisolone 200 mg every 6 hours for any situation in which pulmonary toxicity makes mechanical ventilation likely to be required within 4 hours. If the patient has not had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
5. In life-threating toxicity not improving after 200 mg of methylprednisolone, 1000 mg of methylprednisolone can be administered.

In general, stop corticosteroid use when toxicity improves to a tolerable level. For example, in patients with hypotension, stop methylprednisolone 6 to 12 hours after vasopressors are no longer needed.

Neurological toxicity

1. All patients with neurological toxicities other than somnolence and delirium should get a neurology consult.

2. All patients with significant neurological toxicity should get an MRI of the brain.
3. All patients with significant neurological toxicity should get a lumbar puncture after MRI if it is safe to perform a lumbar puncture.
4. The following patients should receive dexamethasone 10 mg intravenously every 6 hours until the toxicities improve. Note: for seizures administer standard seizure therapies in addition to dexamethasone. For patients already getting higher doses of corticosteroids for CAR-related toxicity, it is not necessary to add dexamethasone 10 mg every 6 hours. Stop dexamethasone as soon as toxicity improves to a tolerable level; the duration of dexamethasone use will need to be determined on a patient to patient basis. Tocilizumab is possibly not effective for neurological toxicity, so it should not be given when patients have isolated neurological toxicity.

    1. Inability of patient to follow simple commands such as “squeeze my fingers”.
    2. Any generalized seizure
    3. Somnolence that is different than normal sleep such as constant sleep or difficult to arouse or any difficulty with airway protection
    4. Ataxia severe enough to preclude ambulation
    5. Disorientation to person or place that persists continuously longer than 24 hours
    6. Neurologic toxicity lasting more than 2 hours that is severe enough to interfere with self-care activities of daily living (ADLs)
    7. Severe agitation or confusion that limit the ability to care for the patient without restraints

15.4 APPENDIX D: INFUSION INSTRUCTIONS

Equipment:

Primary IV tubing (2)

Secondary IV tubing (1)

NS (sodium chloride 0.9%) 250cc bags (2)

IV infusion pump

Gloves

Steps:	**Key Points:**
1. The RN will be informed of the approximate time of cell arrival at the bedside.	
2. Verify the physician orders: - to administer the cells - for the date of administration - for premedication orders - protocol number	• Premeds are acetaminophen 650 mg PO and diphenhydramine 12.5 mg IV.
3. Verify that the protocol consent	
4. Ensure that emergency and monitoring equipment are available in the patient's room: - oxygen - suction - vital sign monitor with pulse oximeter and thermometer	
5. Provide patient education covering infusion procedure, potential complications and associated symptoms to report.	
7. Measure and record baseline vital signs, respiratory and circulatory assessments.	

8. Verify the patency of the patient's IV access.	A central venous access device such as a non-valved PICC line should be used.
9. Hang a primary line of 250cc NS at a kvo rate - **NEW** bag and **NEW** tubing. This **MUST** be ready and infusing **prior** to the cells being delivered to the unit. The patient's primary IV hydration can infuse via a separate lumen while the cells are infusing, but **NO MEDs** should be administered during this time. Have a second bag of 250cc NS and tubing ready as an emergency line.	This will be the dedicated NS line for infusing the cells. Under no circumstances are any other substances to be infused into the line. Cell death occurs quickly – the infusion must be initiated immediately. Do not infuse medication during the cell infusion. If emergency meds must be administered, use the hydration or emergency NS IV line. This will be the emergency IV solution and can be used for medication administration. **Do not use an inline filter for cells.**
10. The primary RN will be notified approximately 10 minutes before the cells arrive on the unit. The cells will be hand delivered to the bedside. It is critical to be at the bedside awaiting the arrival of the cells for infusion.	It is critical to be at the bedside awaiting the arrival of the cells for infusion; have baseline VS, assessment, and IV lines hooked up when the cells arrive. **Cell death occurs as soon as the cells are removed from the laboratory**. Initiate the infusion as quickly as possible.
12. Prior to spiking the cell bag, two RNs will perform the identification procedure. **Both** RNs must have their names charted in the CRIS cellular therapy flow sheet	
13. Infuse the cells by **<u>INFUSION PUMP</u>** or syringe over 20-30 minutes. a. Piggyback the cells into the dedicated NS line; use the backflush technique to prime the line. b. While the cells are infusing, **gently** agitate the bag of cells **every few minutes**. When the cell bag is empty, backflush NS to rinse the bag	This prevents the cells from clumping in the bag.

and infuse this at the same rate as the cells; rinse bag until NS runs clear. c. Note: in some cases cells will arrive from DTM in a syringe. In this case infuse the cells via syringe over 20-30 minutes in the dedicated NS line proximal port, see nursing cellular infusion SOP for further details.	
14. Measure and record VS before and after the cell infusion, q1h x 4, and then q4h after completion of the infusion. a. Assess and document the patient's respiratory and circulatory status post cell infusion.	
15. Documentation: a. After the cells have infused, remove the adhesive backed "cell therapy product" tag from the cell bag and place it on a progress note in the patient's chart. b. Document the cell infusion in CRIS using the appropriate screens.	